

UNIVERSIDADE DE LISBOA FACULDADE DE CIÊNCIAS
DEPARTAMENTO DE BIOLOGIA ANIMAL



**Effectiveness of resilience assessment
tools in different case studies.**

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DOUTORAMENTO EM BIOLOGIA

Especialidade de Biologia Marinha e Aquacultura

2015

UNIVERSIDADE DE LISBOA FACULDADE DE CIÊNCIAS
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tools in different case studies.**

Tese orientada pelo Professor Doutor Henrique Cabral
e pelo Doutor Pierre Jean Valayer,
especialmente elaborada para a obtenção
do grau de Doutor em Biologia
(especialidade de Biologia Marinha e Aquacultura).

‘The greatest glory in living lies not
in never falling, but in rising every
time we fall.’

Nelson Mandela

19 Jul 1918 - 5 Dec 2013

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Acknowledgements

In the first instance, I acknowledge the two supervisors of this work: Prof. Dr. H.N. Cabral and Dr. P.J. Valayer. Without their continuous support this PhD would definitely not have been possible. Beyond sound science and statistics, you have taught me plenty boosting my professional career and also my personal development. Therefore I am forever grateful.

I acknowledge the University of Lisbon, Department of Science for hosting me as their PhD student and providing access to all its facilities. The fellowship of this PhD was granted by the Portuguese National Science and Technology Foundation (reference: SFRH/BD/48402/2008).

I would also like to express my gratitude to the highly professional and extremely productive team of Prof. Dr. H.N. Cabral with whom I had the pleasure to collaborate throughout the years. I definitely learned a lot from working with all of you.

I also thank all my friends, they know who they are. Also my colleagues from Greenloop introducing me to truly innovative concepts such as resilience in management and biomimicry projects.

Last but not least: a warm thank you to my family for their continuous support throughout this big challenge. My parents - ying and yang: altijd paraat - papa strategisch advies - mama pectus pectoris - ik vergeet 1st kan Bio nooit. My brother and sister: Mick - water-sport king - Maxine - Gucci girl with a golden heart. Ook mijn meter Marlene voor de vele ondersteunde mailtjes. Last but not least my little nephews Sebastien en Edouardo growing up so fast - thanks for being you and inspiring me.

Noémie Wouters



List of Papers

This thesis consists of four papers, each corresponding to a chapter from 2 to 5. Wouters N. was the lead author for all publications in cooperation with the co-authors and she was responsible for the data analysis. The field survey of Chapter 2 was also carried out by the first author with support of the colleagues mentioned in the acknowledgments.

Chapter 2

Wouters, N., Serafim, M.P., Baringo, F. C., Valayer, P.J., Cabral, H.N. The impoverished ecological state of a Portuguese coastal lagoon after its artificial opening. Submitted to Brazilian Journal of Biology.

Chapter 3

Wouters, N., Valayer, P.J., Pickerel, T., Vanstaen, K.R., Palmer, D.W., Mills, G., Cabral, H.N. Measuring trends and signals of sustainability in oyster population and production data. Estuarine, Coastal and Shelf Science 2013 130, 231 - 238.

Chapter 4

Wouters, N., Dakos, V., Edwards, M., Serafim, M.P., Valayer, P.J., Cabral, H.N. Evidencing a regime shift in the North Sea using early warning signals as indicators of critical transitions. Estuarine, Coastal and Shelf Science 2015 152, 65-72

Chapter 5

Wouters, N., Serafim, M.P., Valayer, P.J., Kirkup, B.C., Wang, Y.J., Cabral, H.N. Early warning signals as indicators of cyclostationarity in three-species hierarchies. Submitted to Ecological Indicators.

Summary

Systems at times undergo abrupt, often undesirable changes termed regime shifts. Anticipating critical transitions requires the understanding of ecosystem resilience; however establishing operational resilience assessment tools remains a major hurdle assessed by the current study using different case studies. The first analysis, aiming at analysing the recovery time of macrobenthos in a coastal lagoon, evidenced the system previously underwent a regime shift. Only one taxon showed a significant recovery in response to the management action with the objective of improving the status of the lagoon. A lag in response time, *hysteresis*, constituted a major challenge for sound restoration management. The following studies assessed recently proposed statistical signals capable of anticipating shifts, early warning signals (EWS) as resilience assessment tools. First EWS were calculated on oyster landings with an oscillating trend and also catch rates characterized by an irreversible decline. In both cases the tools accurately reflected the stability of the respective trends of the case studies. Three bottlenecks related to the use of EWS were identified: the quality of the time series, EWS behaviour in cyclic dynamics and the influence of the turnover rate of the measures upon which EWS are calculated. Next EWS were applied to phytoplankton data of a regime shift in the North Sea, as an example of a biotic signal with fast turnover rate. In one case, the resilience loss was detected 12 years prior to the shift. The high-resolution data enabled advanced methodological testing confirming the robustness of the indicators and their trends. Finally a model was used to assess whether EWS might indicate a loss of cyclostationarity. It simulated three species in a competitive cycle: the first scenario oscillated stably while the second scenario was programmed with a gradually decaying resilience. Indeed EWS can be used to detect instabilities in cyclic phenomena, however the choice of the indicator remains essential. While hurdles do exist, we conclude that overall EWS are promising resilience assessment tools, and particularly recommendable considering the ease of their calculation.

Keywords

resilience, regime shift, recovery time, early warning signals, cyclostationarity

Resumo

Ecosistemas são por vezes sujeitos a reconfigurações estruturais e funcionais súbitas indesejáveis. Antecipar essas mudanças de regime requer o conhecimento da resiliência dos ecossistemas; no entanto a quantificação da mesma consiste dum desafio. Esta dissertação foca-se na avaliação da eficácia de ferramentas da resiliência fazendo uso de diferentes casos de estudo. O primeiro estudo, como objetivo de avaliar o tempo de recuperação da comunidade macrobentónica duma lagoa costeira, revelou uma mudança de regime anterior à amostragem. Apenas um *taxon* mostrou uma recuperação significativa em resposta a medidas de gestão destinadas a melhorar a qualidade do estado ecológico da lagoa. O atraso no tempo de reposta da comunidade de macroinvertebrados bentónicos, *hysteresis*, dificultou uma gestão efetiva. Os estudos seguintes tiveram como objetivo avaliar a eficácia de indicadores estatísticos, os sinais de alerta precoce caracterizados pela sua capacidade de detectar uma perda de resiliência. Estes foram aplicados em séries-temporais de produção de ostras com uma tendência oscilante e taxas de captura caracterizadas por um declínio irreversível. Nos dois casos, as ferramentas refletiram a estabilidade e a instabilidade das tendências, respetivamente. Em relação à sua leitura, três desafios foram identificados: a influência da qualidade da série temporal; o comportamento dos indicadores nos sistemas cíclicos; e a influência da taxa de inversão das medições sobre as quais os sinais são calculados. O terceiro estudo fez uso de parâmetros fitoplanctónicos sendo um sinal biótico caracterizado por uma taxa de mudança elevada. Num dos indicadores a tendência indicou que a alteração de regime pôde ser detectada com 12 anos de antecedência. Além do mais a elevada resolução da série-temporal permitiu metodologias de experimentação avançadas confirmando a robustez dos indicadores e das tendências. No último estudo foi utilizado um modelo de três espécies interagindo num ciclo competitivo que avaliou a capacidade dos sinais de identificar uma perda de resiliência nos fenómenos cíclicos. Nos dois cenários, o primeiro estável e o segundo modelado a perder gradualmente a sua resiliência, os sinais de alerta precoce identificaram efetivamente a estabilidade do sistema e a degradação da sua ciclicidade.

Palavras chave

resiliência, mudanças de regime, hysteresis, sinais de alerta precoce, ciclicidade

Resumo Alargado

As pressões antropogénicas às quais os ecossistemas estão sujeitos, têm vindo a aumentar nas últimas décadas. A resposta dinâmica dos ecossistemas ao aumento de pressões tal como a eutrofização e a poluição são lineares até se atingir um nível crítico ou ‘threshold’ após o qual o comportamento tornam-se descontínuas. Conceptualmente, a estabilidade dos ecossistemas é frequentemente representada como uma paisagem ondulada, representando o domínio de atração, dentro do qual a posição duma bola reflete o (des)equilíbrio da mesma (figure 1.1). Sujeita a pequenas perturbações, a bola volta rapidamente ao seu estado original, no entanto, grandes perturbações podem forçar o ecossistema para um estado de equilíbrio dinâmico alternativo. A transposição de tais pontos críticos implica reconfigurações súbitas ao nível estrutural e funcional, denominadas mudanças de regime. Estas são frequentemente associadas a perdas de serviços e bens e nalguns casos o retorno ao estado ecológico original é pouco provável. Neste âmbito, a identificação e antecipação de mudanças de regime são extremamente valiosas para uma gestão efetiva que assegure a sustentabilidade dos ecossistema. Neste sentido, a quantificação da resiliência, definida como a capacidade dum determinado ecossistema de restabelecer o seu próprio equilíbrio após um distúrbio, é essencial. De facto, o conceito de resiliência tornou-se fundamental na gestão de ecossistemas. No entanto, apesar do seu enquadramento teórico estar bem estabelecido, as ferramentas específicas de quantificação são limitadas. Esta dissertação foca-se na análise da eficácia de ferramentas de avaliação da resiliência fazendo uso de diferentes casos de estudo. O primeiro estudo tinha com objetivo a avaliação do tempo de recuperação duma comunidade macrobentónica numa lagoa costeira. No entanto, esta revelou um estado de degradação avançado, evidenciando a ocorrência duma mudança de regime antes da amostragem ser efectuada. Apenas um *taxon* teve uma recuperação significativa em resposta a medidas de gestão destinadas a melhorar a qualidade do estado ecológico da lagoa. Uma das conclusões chave foi que um atraso no tempo de reposta, *hysteresis*, constitui um obstáculo maior na recuperação efetiva do ecossistema. No caso dum determinado limite ser ultrapassado pode tornar-se necessária a implementação de medidas de gestão mais drásticas e com elevados custos. Por outro lado, o estudo mostrou que a eficácia da avaliação da resiliência é influenciada pelo planeamento do programa de monitorização: uma amostragem

contínua e com alta resolução espacial é deste modo essencial.

Uma nova abordagem proposta recentemente pela comunidade científica sugere o uso de indicadores estatísticos com a capacidade de detectar instabilidades nos ecossistemas para tentar antecipar mudanças de regime. Estes indicadores denominam-se sinais de alerta precoce. Estas ferramentas, exploráveis através do estudo de séries-temporais, têm sido validadas em diferentes áreas do conhecimento, incluindo medicina, economia e biologia. Os estudos que compõem esta dissertação focam-se na análise dos sinais de alerta precoce em múltiplos casos de estudo. Em primeiro lugar foram aplicados a dois tipos de dados de ostras provenientes de diferentes zonas geográficas: dados de produção e taxas de captura. Uma análise de tendência revelou oscilações e um declínio irreversível das taxas de produção e captura, respectivamente. Em ambos os casos os sinais de alerta precoce refletiram precisamente a estabilidade e a instabilidade das tendências. No entanto, três desafios surgiram relativamente à sua utilização: primeiro, a qualidade das séries-temporais; segundo, o seu comportamento em mudanças de regime em sistemas caracterizadas por uma dinâmica cíclica; e por último, a influência da leitura dos sinais em relação à taxa de inversão das medições sobre as quais são calculados. O terceiro estudo fez uso de parâmetros fitoplanctónicos como sinais bióticos com uma taxa de mudança diária, no âmbito dum dos maiores programas mundiais de monitorização, cobrindo uma mudança de regime no Mar do Norte nos anos oitenta. No caso de um dos indicadores, a alteração de regime foi detectada com 12 anos de antecedência. Confirmou-se também que a resolução da série-temporal contribui para a eficácia dos sinais de alerta precoce. Neste estudo a resolução dos dados, compostos por amostragens mensais, permitiu o avanço das metodologias de experimentação através da avaliação da robustez e da significância dos indicadores a das suas tendências. Um constrangimento da metodologica no cálculo dos sinais de alerta precoce é a dependência estatística da janela temporal sobre o qual é baseado o seu cálculo. No entanto, esta investigação revelou que uma estratégia efetiva para ultrapassar esta dificuldade é a divisão da série-temporal em blocos independentes. Tendo em conta os obstáculos, a utilização dos sinais de alerta precoce é altamente recomendada visto que o seu cálculo é relativamente simples fazendo uso de ferramentas disponíveis online.

O último objectivo desta dissertação focou-se na avaliação da capacidade dos sinais de alerta precoce de identificar uma perda de ciclicidade. A ciclicidade dinâmica é comum nos ecossistemas,

no entanto, poucos estudos fizeram uso dos sinais de alerta precoce na sua perda de resiliência. Utilizou-se um modelo de três espécies interagindo num ciclo competitivo em dois cenários: o primeiro consistiu numa oscilação estável, enquanto que no segundo cenário a interação das três espécies foi gradualmente degradada. A principal conclusão foi que os sinais de alerta precoce têm efetivamente a capacidade de detetar instabilidades nos fenómenos cíclicos, no entanto, a escolha do tipo de indicador é crucial.

Em suma, a chave da avaliação da resiliência está na capacidade de antecipar quando um ecossistema está à beira da mudança de um estado de equilíbrio para um outro. É igualmente importante avaliar se e quando um sistema em mudança pode eventualmente voltar o seu estado original e compreender quais são os parâmetros que conduzem a tal ciclo de *hysteresis*. Além disso, os sistemas biológicos estão frequentemente sujeitos a ciclos naturais, globalmente estáveis, mas nos quais as pressões dinâmicas externas ou internas podem resultar numa perda de estabilidade.

As publicações resultantes desta dissertação sublinham a relevância do desfasamento em termos de tempo de resposta, *hysteresis*, como também a importância dos indicadores estatísticos com a capacidade de antecipar mudanças de regime, fazendo uso de análises de séries-temporais e modelação. Nos diferentes casos de estudos, dificuldades como a robustez e a qualidade das séries-temporais são discutidos a par da avaliação crítica da eficácia dos sinais de alerta precoce. No último capítulo são ainda sugeridas possíveis linhas de investigação futura, tendo em conta as questões levantadas pelo presente estudo. Nomeadamente, foi sublinhado a necessidade de aumentar os casos de estudos nos estuários, e em particular, recomenda-se uma análise mais detalhada dos diferentes habitats (intertidal, lagoas costeiras, estuários, barragens de corais, etc). O desenvolvimento de estudos marinhos e estuarinos contribuirá para o estabelecimento duma base de dados refletindo as tendências dos sinais de alerta precoce nos diferentes habitats. Por último, poderia também ser efectuada uma classificação dos mesmos habitats consoante o seu nível de resiliência, o que poderá resultar na elaboração duma escala universal de resiliência. Finalmente, em relação à aplicação dos sinais de alerta precoce na gestão de ecossistemas e recursos, a primeira etapa consiste na familiarização dos decisores e gestores do uso dos sinais de alerta precoce, sublinhando as suas vantagens, nomeadamente a de que o seu cálculo ser relativamente simples. Incorporar os sinais de alerta precoce nos planos de monitorização pode ser vantajoso fazendo uso de amostra-

gens de longa duração e com alta resolução espacial e de dados em tempo real usando por exemplo satélites e boias.

Chapter 1

Introduction.

Prior art and objectives

One of the first biologists to mention the importance of nonlinear dynamics in ecology was Crawford Stanley Holling recognising that minor changes in ecosystems forced away from their local equilibrium, can induce large overall structural changes (Holling 1973). By defining dynamically stable conditions in his publication on the resilience and stability of ecological systems, he significantly influenced integrative theories within ecology by introducing new concepts such as local equilibrium and basin of attraction. Illustrated with case studies e.g. the fish stock depletion in the Great Lakes (USA), Holling made theoretical suggestions on the behaviour of systems distinguishing stable, unstable equilibria and cycles. Holling also emphasised other ecosystem properties essential for analysing ecological processes: lags in response time, thresholds and the evolution of spatial elements over time. Holling was one of the first authors to formally introduce the concept of resilience as a measure of the persistence of ecosystems and of their ability to absorb disturbances while maintaining similar structural relationships between populations or state variables. After this formal definition, resilience became a central concept in the management of natural ecosystems. Resilience encompasses two fundamentally different but rarely distinguished processes: resistance and recovery (Côté and Darling 2010). For a given structural or functional parameter, resistance is defined as the amount of a given pressure that can be applied without deterioration in status, expressed by a specific measure. When the pressure is removed, the status may not improve promptly introducing a lag: ‘type I *hysteresis*’ (Elliott *et al.*, 2007). Given time, though, recovery might occur, although it does not necessarily imply a return to original levels. The degree of recovery, compared to the original status is defined as ‘type II *hysteresis*’ where complete resilience results in a return to the original level, and partial resilience is a return to a different (lower or higher) level (Elliott *et al.*, 2007). While its conceptual approach and theoretical terms are well established, the actual measurement is fraught with difficulties and resilience remains poorly quantified especially in the marine environment (Côté and Darling 2010).

To assess recovery time requires an understanding of the current and previous state of the ecosystem supported by empirical evidence (Elliott *et al.*, 2007). Ecosystems may respond gradually to external pressure, while in some cases abrupt changes are displayed (Mollman and Diekmann

2011). One way of assessing the state of an ecosystem and its potential recovery is by analysing the evolution of univariate metrics, such as diversity and abundance, and their relationship to environmental variables (Schaffner 2010). Macrobenthos characterized by only limited migration can provide useful insights into the community response following disturbances (Buss and Jackson 1979; Burrow *et al.*, 1998). Generally frequent environmental fluctuations result in resilient macrobenthic communities: for example the resistance of the composing macrobenthic communities of coastal lagoons is considered high, closely linked to the high variability in for example salinity and oxygen content (Munari *et al.*, 2005). However especially in intermittently open/closed lagoons, excessive nutrient loading might result in an irreversible regime shift to a macro-algae dominated ecosystem (Pereira Coutinho *et al.*, 2012; Viaroli *et al.*, 2008). To prevent eutrophication, a common management practice in Portugal consists of the artificial opening of the lagoons (Cancela da Fonseca *et al.*, 1999; Costa *et al.*, 2003; Carvalho *et al.*, 2011). Once connected to the sea, flushing will prevent eutrophication and drain the alluvial plains for agriculture (Freitas *et al.*, 2008).

Regime shifts like eutrophied coastal lagoon shifting to being entirely dominated by algae, are defined as abrupt changes on several trophic levels leading to ecosystem reconfiguration between alternative states (Andersen *et al.*, 2009). They might have important socioeconomic influences as good and services are likely to be different under the new regime, as demonstrated in marine studies on shifts between alternative states in coral reefs and kelp forests (Boettiger *et al.*, 2013, Hughes *et al.*, 1994; Konar and Estes 20003). The development of management strategies and operational tools to mitigate or to avert critical transitions is therefore crucial to ensure the sustainability of ecosystems and their biotic resources (Dakos and Hastings 2013). Regime shifts are considered abrupt in the sense that the timespan of their occurrence is relatively short (1–2 years) in comparison to the duration of the different states, often decadal to multi-decadal (Mollman and Diekmann 2011). Numerous examples have been detected in terrestrial, freshwater and marine ecosystems and in Europe a comparative analysis of shifts induced by climate change and overfishing in different marine regions including the Bay of Biscay, and the North, Baltic, Black and Mediterranean Sea, the Bay of Biscay, is available (Mollman *et al.*, 2011). Conceptual models describe different types of critical transitions by representing the equilibrium state visualised by the ecosystem's response e.g. a structural or functional parameter as a function of an altering external condition

e.g. nutrient loading (Elliott *et al.*, 2007). In some cases a linear response occurs, others show the equilibrium state suddenly crossing a non-catastrophic threshold and finally the equilibrium curve might also show a so called backward-folding where, for a specific environmental condition, two alternative stable states exist (Scheffer *et al.*, 2009). The latter implies the existence of two alternative stable states with a bifurcation point, where a minor alteration drives the system across a boundary causing a large transition (Mollman and Diekmann 2011). Small perturbations might also induce large transitions in the absence of true bifurcations, provided that the system is highly sensitive in a certain range of conditions (Scheffer *et al.*, 2009). Finally one other class of important bifurcations are those that mark the transition from a stable equilibrium to a cyclic or chaotic attractor (Scheffer *et al.*, 2009).

Different numerical methods exist to detect shifts including multivariate techniques such as ordination (Mollman and Diekmann 2011). Recent research has focused on statistical tools that have the capacity to anticipate critical transitions: early warning signals (EWS). The dynamics of a variety of systems, ranging from financial markets to neurological disorders such as epilepsy, are characterized by generic properties near transitions known in the dynamical systems theory as ‘Critical Slowing Down’ (CSD). It refers to a universal law related to the characteristic return time of systems near thresholds (Wissel 1984). Near a critical point, the recovery rate after disturbance takes longer (Vervaart *et al.*, 2011). Even without insights in the mathematical background of the theory, the concept of CSD can be easily understood by a visual representation of the basin of attraction as a stability landscape like a ball in a basin: resilient ecosystems have a deep basin and when stressed, their recovery is fast. On the other hand if the basin is shallow (brittle ecosystem) and external forcing occurs, the ball does not only return slower to its original state but is also more readily flipped out of the basin: the risk of transition is high (Scheffer 2010) (figure 1.1).

In summary, after a temporary disturbance, the more swiftly the system returns and the less it fluctuates, the more stable it is (Holling 1973). This concept is applied in the management of terrestrial ecosystems namely rangelands: when the grazing pressure becomes too intense, the risk of being overrun by weeds is high and managers attempt to obtain a warning of an impending transition by monitoring how the time to extinction of small outbreaks of the weed changes as the grazing rate increases (Chisholm and Filotas 2009). In some cases (e.g. oysters) recovery rates can

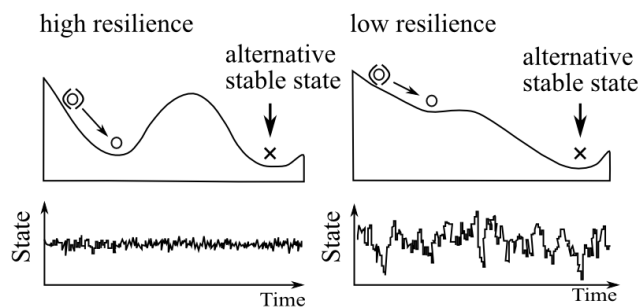


Figure 1.1: Conceptual image of high vs low resilience landscape

take up several years, and even decades (Buestel *et al.*, 2009). Systematically testing for recovery rates as in the example above is therefore often difficult especially for complex systems (Scheffer 2010). Another approach to identify CSD relies on statistical parameters that will fluctuate and change in a predictable way. A variety of leading indicators, EWS, exist and can be extracted by time series analysis (Scheffer *et al.*, 2009; Ives and Dakos 2012; Drake and Griffen 2010). A first tool frequently used is autocorrelation, the increase of which is characteristic of a CSD (Lenton *et al.*, 2012a,b). Subsequent states in a time series will become more alike, so the short-term memory (=autocorrelation at low lags) increases (Scheffer *et al.*, 2009; Scheffer 2010). Slow recovery is often associated with further accumulated perturbations resulting in an increasing variance of the fluctuations prior to a transition (Dai *et al.*, 2012). Increasing variance is a second parameter often used as EWS. Thirdly particular spatial patterns arising before a critical transition might also serve as EWS (Scheffer *et al.*, 2009). Spatial assessments consider systems as numerous coupled units influencing each other states: for example the persistence of species in a fragmented landscape is determined by the presence of the same species in neighbouring patches allowing recolonisation (Bascompte and Solé 1996). When these self-organised regular patterns are subjected to habitat fragmentation, the structural properties of the resulting landscape changes in a non-linear way with a general tendency towards increased spatial coherence among units prior to extinction (Bascompte and Solé 1996). Although classes of spatial EWS have been observed across systems, there is no ‘one-size-fits-all’ when interpreting spatial patterns announcing critical transitions (Scheffer *et al.*, 2009). A fourth method used as EWS is a power spectrum analysis reflecting changes in the complete spectral properties of a time series prior to a transition (Dakos *et al.*, 2012). Spectral

analysis might be a promising tool especially for cyclic phenomena omnipresent in nature and expected to also display leading indicators when forced to their border of attraction (Scheffer *et al.*, 2009). In this case the power of spectral densities would shift to lower frequencies.

In biology, the existence and validity of EWS as leading indicators of regime shifts are discussed in recent publications in high impact journals (Scheffer *et al.*, 2009; Scheffer, 2010; Dai *et al.*, 2012; Drake and Griffen, 2010; Lenton, 2011; Wang *et al.*, 2012). Numerous examples are at hand in paleoclimate analysis, lake eutrophication and arid ecosystems desertification (Rietkerk *et al.*, 2004; Dakos *et al.*, 2008; Dakos *et al.*, 2011; Lade and Gross, 2012; Wang *et al.*, 2012; Biggs *et al.*, 2009; Brock and Carpenter, 2012). Both experimentally and in models, EWS have been observed; for example, in a generalised fishery model EWS indicated the shift from a high-piscivore, low-planktivore to a low-piscivore, high-planktivore regime (Lade and Gross, 2012). Laboratory populations of the zooplankton *Daphnia magna* subjected to declining food provision have also exhibited CSD (Drake and Griffen 2010). Finally EWS were also observed in the laboratory in yeast populations subjected to the Allee effect by increasingly dissolving the population to eventually collapse (Dai *et al.*, 2012). However application of EWS to marine ecosystems and datasets are still rare. Lindegren *et al.* (2012) estimated EWS in a large-scale reorganisation of the Baltic Sea ecosystem using two indicator copepod species but found no clear patterns. Litzow *et al.* (2008) used a measure of community composition on trawl survey data and observed increased spatial variability accompanying the reorganisation of two continental shelf ecosystems in the North Pacific (Gulf of Alaska) and the North Atlantic (Scotian Shelf), while Litzow *et al.* (2013) compared collapsing and non-collapsing models of Crustacean fisheries. Despite this work, we still lack understanding of the applicability of EWS as tools for anticipating regime shifts in marine ecosystems. In short EWS have been applied to a variety of signals ranging from abiotic measures such as paleo-temperature records, to biotic parameters the latter including different trophic levels ranging from macro to microorganisms and also models. Their widespread use resulted in the availability of extensive methodological guidelines including flowcharts and online toolboxes (Dakos *et al.*, 2012, <http://www.early-warning-signals.org/>). Care is needed in the data processing prior to EWS calculation and also in the interpretation of the results as CSD does not always associate with rapid shifts, and vice-versa (Dakos *et al.*, 2012; Lenton *et al.*, 2012 a,b; Boettiger *et al.*, 2013). Specific

methodological hurdles include the robustness of the indicator themselves and the statistical significance of their trend, that instead of the absolute EWS values, is ultimately used as indication of CSD (Lenton *et al.*, 2012). The length of the time series might also play an important role: EWS readings in shorter time series are more challenging (Mollman and Diekmann 2011).

The applicability and utility of EWS to real ecosystem management is assessed by different criteria (Lindeberg *et al.*, 2012). A crucial issue is a timely warning: how far from the transition are EWS observed: if the trend increases only briefly before, it is unlikely that the shift can be anticipated or avoided like in the case of a transition from oligotrophic to eutrophic lakes, which could have been avoided by employing simple remedial measures (Donangelo *et al.*, 2010; Chisholm and Filotas, 2009). Also using a fisheries model of a regime shift, the increase in the EWS indicators only occurred once the regime shift had already initiated, too late for management to avert a shift (Biggs *et al.*, 2008). This PhD dissertation assesses the applicability of resilience assessment tools in different case studies. The first paper uses recovery time after a management intervention to evaluate the state of a small lagoon along the South-West coast of Portugal by assessing the spatiotemporal evolution of its macrobenthic community. The following studies apply EWS to biotic variables including macrobenthos and phytoplankton data and a model under different dynamics including irreversible declines and cyclic trends. The utility and application of EWS in a management setting is assessed, with special attention to the robustness and significance of the findings. Finally a model approach is deployed to assess how stability and EWS evolve in a system where three biotic components are interacting circularly.

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Chapter 2

The impoverished ecological state of a Portuguese coastal lagoon after its artificial opening.

Abstract

Coastal lagoons are ecological islands subjected to frequent environmental fluctuations. Their conservation is vital to safeguard their socioeconomic value but also their unique biotic diversity. This paper evaluates the spatiotemporal evolution (2-way analyses of variance) of the macrobenthos in a Portuguese lagoon in six stations monitored frequently over a 9 month period covering two short artificial openings to the sea. The assessment revealed the poor ecological state of the lagoon with a drastic impoverishment since the 80'ties. The management practice of connecting the lagoon to the sea, proven effective in other cases, did not result in restoration. Instead it represented an additional physical disturbance to the macrobenthic community and only the chironomidae (Diptera) were able to re-establish their population. The relationship of the benthic community to environmental variables (temperature, salinity, dissolved oxygen, pH, Eh and Secchi depth) was also examined using multivariate analysis and revealed temperature and salinity as the most important parameters structuring the community. The poor ecological status of the lagoon stresses the urgent need of implementing a revised management plan. A more frequent and a more prolonged opening with a widening of the inlet channel while taking into account the species life cycles, could be considered to allow effective flushing and the colonisation of marine *taxa*.

Keywords

coastal lagoon, recovery, macrobenthos, insect larvae, resilience, restoration

Resumo

As lagoas costeiras são consideradas ilhas ecológicas sujeitas a flutuações ambientais frequentes. A sua conservação é vital para salvaguardar o seu valor socioeconómico tal como a sua biodiversidade única. Nesta publicação a evolução espacio-temporal (análise de variância a 2 factores) da comunidade macrobentónica numa lagoa costeira da costa Portuguesa é avaliada: 6 estações foram frequentemente amostradas durante um período de 9 meses cobrindo duas aberturas artificiais da lagoa para o mar. Os resultados revelaram um estado ecológico degradado incluindo uma redução drástica da biodiversidade desde os anos oitenta. A gestão que consistia em abrir artificialmente a lagoa com objectivo de estabelecer uma conexão para o mar, provada efectiva noutros casos, não resultou numa renovação desta lagoa. Pelo contrário, a medida de gestão representou uma perturbação física suplementar para comunidade macrobentónica sendo que os Chironomidae (Diptera) foram o único taxon que conseguiu re-estabelecer as suas populações. A relação da comunidade macrobentónica com os parâmetros ambientais (temperatura, salinidade, oxigénio dissolvido, Ph, condutividade, profundidade Secchi) foi investigada fazendo uso de uma análise multivariada que revelou que a temperatura e salinidade foram os dois parâmetros mais importantes para estruturar a comunidade. O estado ecológico degradado põe ênfase na necessidade urgente numa revisão estratégica do plano de gestão da lagoa. Uma abertura mais frequente e mais prolongada incluindo um alargamento do canal de abertura tendo em conta o ciclo da vida das espécies residentes deve ser considerada, facilitando uma descarga mais eficiente da lagoa e permitindo potencialmente a colonização de *taxa* marinhas.

Palavras-chave

lagoa costeira, comunidade macrobentónica, recuperação, resiliência, restauração, larvas de insetos

2.1 Introduction

Coastal lagoons form a transient barrier between land and sea separated by a wide ridge and communication is established during extreme high tides, stormy weather or by waves, when seawater washes over into the lagoon (Suzuki *et al.*, 2002). The frequent environmental variability they are subjected to results in naturally stressed ecosystems (Pérez-Ruzafa *et al.*, 2013). Generally environmental fluctuations generate resilient communities and the resistance of the composing macrobenthic communities of lagoons is considered high (Munari *et al.* 2005). Some coastal lagoons are found to be highly productive, supporting a diverse benthic community serving as food resource for fish (Gamito *et al.*, 2003). On the other hand, others submitted to excessive anthropogenic stress might undergo regime shifts resulting in an extremely low diversity and a dominance by detritivores (Gamito 2006). Anthropogenic pressures include fishing and other urban, industrial and agricultural activities (Gamito 2008). Eutrophication also continues to be a major threat; at times thresholds are exceeded with excessive nutrient loading resulting in an irreversible shift to a macroalgae dominated ecosystem (Pereira Coutinho *et al.*, 2012; Viaroli *et al.*, 2008). In this case the seasonal and inter annual variation combined with spatial variability of coastal laggons make the implementation of an effective management framework particularly challenging (Pusceddu *et al.* 2007). For example the restoration of the recruitment potential in degraded lagoons is difficult (Beer and Joyce 2013).

Resistance and recovery time are concepts essential for effective management, and require an assessment of the current and previous state of the ecosystems supported by empirical evidence (Elliott *et al.*, 2007). In the North Atlantic surveys of coastal lagoons have been published in the grey literature, but data deficiency continues to challenge the successful management of these environments and the preservation of the valuable ecosystem and socio-economic services they represent (Beer and Joyce 2013). On the other hand, in Great Britain, an overview of a wide variety of lagoons allowed the establishment of general guidelines to maintain diversity (Bamber *et al.*, 1992). In Brazil the data of long-term monitoring of physical, chemical and biological variables was used to test for the degree of temporal coherence among 18 tropical coastal lagoons in search of common patterns and mechanisms (Caliman *et al.*, 2010). The Portuguese coast is characterized by several small coastal lagoons and the most common management practice consists of their artificial

opening during the dry season to promote water exchange and assure its water quality (Cancela da Fonseca *et al.*, 1999; Costa *et al.*, 2003; Carvalho *et al.*, 2011). A single narrow entrance is created, and natural closure occurs after a variable number of days. Most coastal lagoons receive water and organic matter from their basin during the winter (Bamber *et al.*, 1992). When opened, water renewal prevents eutrophication and drains the nearby alluvial plains for agriculture (Freitas *et al.*, 2008). However when the connection with the sea is established, radical alterations in physico-chemical conditions take place with the reduction of the water volume and the salt water influx (Suzuki *et al.*, 2002). Little attention has been paid to the effect this might have on the spatiotemporal evolution of the macrobenthic community (Costa *et al.*, 2003; Carvalho *et al.*, 2011). Also the dynamic sedimentary conditions during the opening are relevant as in deposition of dredged material, where they might affect resident biota (Angonesi *et al.*, 2006).

The timing and duration of the opening for example are essential as the structure of the macrobenthic communities might be affected, as extended periods of closure have been associated with anoxia and biotic stress (Quintino 1988). One way of assessing the state of an ecosystem and its potential recovery is by analysing the evolution of univariate metrics, such as diversity and abundance, and their relationship to environmental variables (Schaffner 2010). Furthermore identification of key factors controlling the rates of recovery from different kinds of anthropogenic disturbances is important for ecosystem restoration efforts (Elliott *et al.* 2007). This paper contributes to the progress of research on North Atlantic lagoon systems by assessing the state of a small lagoon along the coast of Portugal. Secondly the spatiotemporal evolution of the macrobenthic community is assessed specifically in response to the current management practice of its artificial opening.

2.2 Material and Methods

2.2.1 Site and sample design

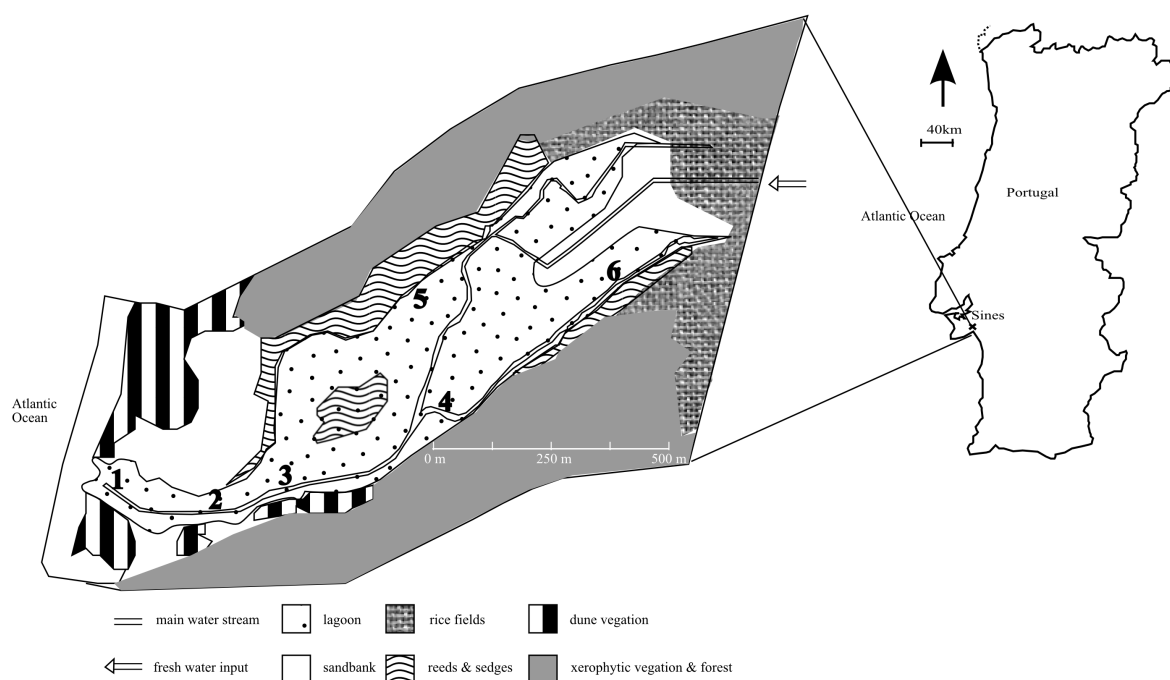


Figure 2.1: Location of the lagoon of Melides along the coast of Portugal with detail of the sample sites (1-6) and an indication of the major vegetation types

The sampling was carried out in Melides, a small coastal lagoon situated 20 km North of Sines on the South-West coast of Portugal (figure 2.1). It occupies an elongated-flooded surface of 0.4 km² and has an average depth of 2 m. The freshwater input is located 14 km upstream and the lagoon receives water from a basin area of 63 km². Most of the time the lagoon is separated from the sea by a sand bar. In 2008, under the request of local rice-farmers, Melides was artificially opened twice, with a two-week interval. After each opening, the lagoon was subjected to the tidal regime of the sea for an average of five days, whereafter natural closure occurred (Freitas *et al.*, 2008). To capture both temporal and spatial variability of the macrobenthic community, six stations (1-6) were sampled from February until September (figure 2.1 - table 2.1). The stations were located at an increasing distance from the sea: 40, 250, 400, 800, 1000, 1300 meters for 1, 2, 3, 4, 5 and 6 respectively. Eight sampling campaigns (a-h) were carried out (table 2.1). Macrobenthic samples

Table 2.1: Lagoon status and sampling time

Sample Code	Lagoon Status	Month
<i>a</i>	<i>prior to opening</i>	<i>February</i>
<i>b</i>	<i>opening</i>	<i>April</i>
<i>c</i>	<i>opening</i>	<i>April</i>
<i>d</i>	<i>after closure</i>	<i>May</i>
<i>e</i>	<i>after closure</i>	<i>June</i>
<i>f</i>	<i>after closure</i>	<i>June</i>
<i>g</i>	<i>after closure</i>	<i>July</i>
<i>g</i>	<i>after closure</i>	<i>September</i>

were taken with a Van Veen grab (surface: 0.05 m²). They were handled standardly being sieved through a 500 μ m square mesh. The retained material was fixed in 4 % buffered formalin stained with Rose Bengal. Species were determined up to the lowest taxonomic level possible. For each station a multi-parametric sonde was deployed to obtain salinity (measured in PSU), temperature ($^{\circ}$ C), dissolved oxygen (D.O., mg/l), pH and Eh (reduction potential, mV) at the bottom. The water transparency was empirically assessed by the Secchi dish method (SD, m) (Chambers and Kaiff, 1985). In the first sampling campaign, additional grab samples were collected for sediment grain size assessment. Grain-size analysis was undertaken by standard sieving on sediment coarser than 63 μ m and the undersized fraction was studied by using a Malvern laser particle analyser. The classification was done according to Larssonneur (1977). For station 5 no environmental data was available.

2.2.2 Data analysis

Data analyses were carried out using the R-software version (R. development core team 2014). A log transform (+1) was applied on the densities and used for all further statistics, to obtain a log-normal distribution (Le Pape *et al.*, 2004). The means of the log transforms and their respective standard errors were represented graphically on a log-scale (Le Pape *et al.* 2004). To test for significant spatiotemporal differences in the evolution of the macrobenthic community two-way analyses of variance (2-way anova) with the factors ‘Time’ (sample periods a-h) and ‘Station’ (sample stations 1-6) were used (Venables and Ripley 2002). The test was run for each taxon individually.

Likewise 2-anovas were performed on the abiotic variables. In case of significant spatiotemporal differences on both parameters combined a trend surface approximated by a polynomial expansion of the control points, was fitted covering the entire lagoon over time. The contour plots depict the variation of the time of the lagoon system. The polynomial function was generated by the method of least squares ensuring minimum deviations (Venables and Ripley 2002):

$$f(x, y) = \sum_{r+s \leq p} a_{rs} x^r y^s \quad (2.1)$$

with:

p = order of the polynomial, here $p = 3$

a_{rs} = polynomial coefficients calculated by .R function: `surf.ls`

x^r = sample periods

y^s = distance from the sea (equivalent sample stations) To test for significant differences in faunal density associated to granulometry, a one-way anova was performed on each taxon with categorised granulometric data. The following three classes were considered: sand percentage $>90\%$, between 70 and 90% , and $< 30\%$.

2.2.3 Multivariate analysis

A principal component analysis (pca) was carried out on the covariance matrix of environmental variables and log-biotic density, and represented in a biplot (Gabriel 1971). Prior to running the pca, redundancy was assessed by Pearson correlation coefficients: for pairs with a significantly high correlation one of the variables was discarded (Tabachnick and Fidell 2007). In addition a multivariate analysis of variance (manova) was used to test for significant influence of the environmental variables on the macrobenthic community as a whole (Tabachnick and Fidell 2007).

2.3 Results

2.3.1 Spatiotemporal variability of benthic community

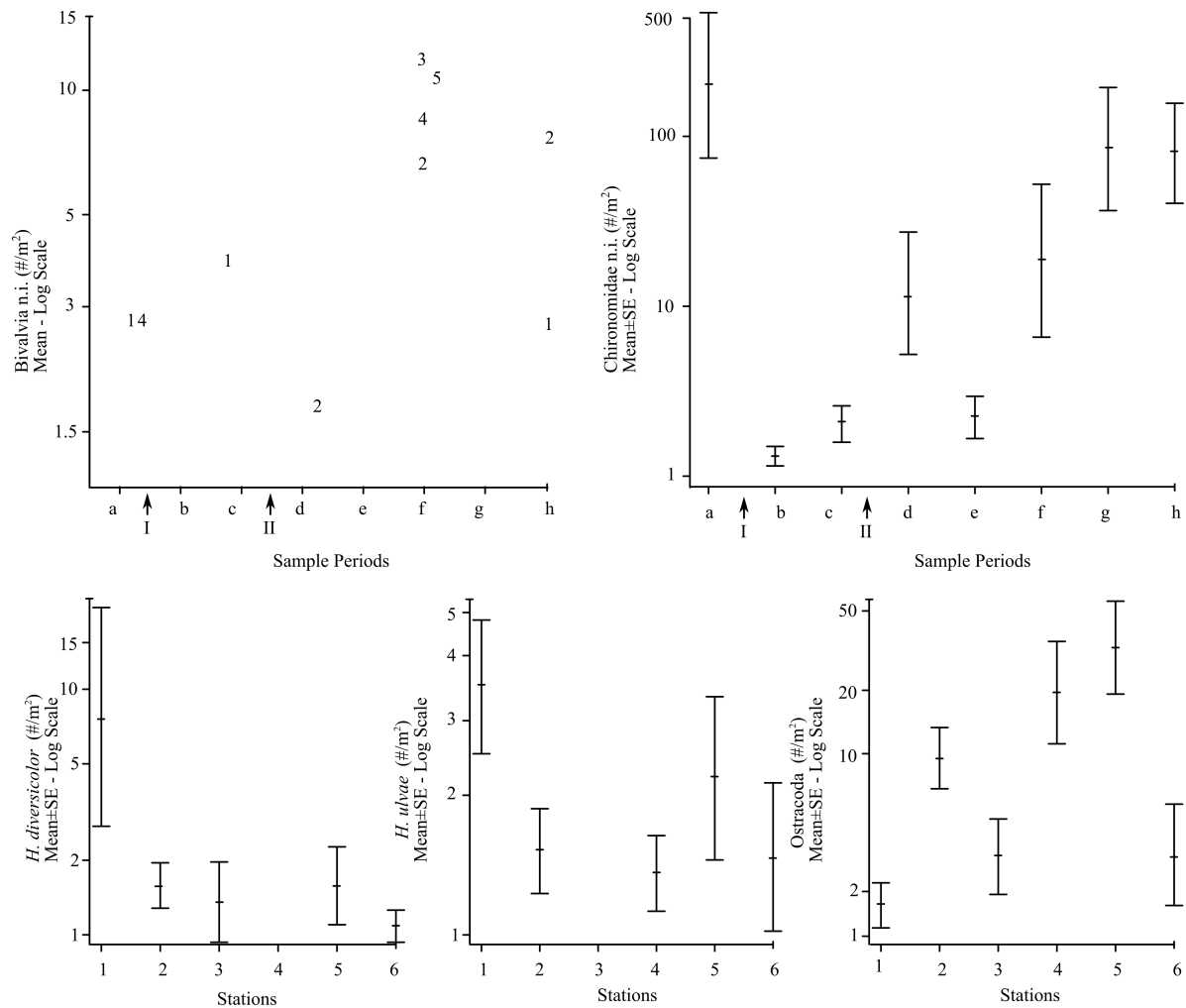


Figure 2.2: Significant results of 2-way anova's (factor 'sample and station') on faunal densities(#/m² mean ±SE) presented on a logarithmic scale: spatiotemporal evolution of bivalvia n.i. (stations 1:5), chironomidae over sample periods and *H. ulvae*, *H. diversicolor* and ostracoda in stations (p-values, MS and F see table 2.2)

The seven *taxa* encountered were: chironomidae n.i., *H. ulvae* (Pennant 1777), *H. diversicolor* (Muller 1776), *L. rugicauda* (Leach 1814), Bivalvia n.i., oligochaeta and ostracoda. Oligochaeta only occurred once in low abundance two weeks after the second opening (6#/m² in d). The 2-way

anova's revealed that chironomidae varied significantly only over time ($p=***$, MS and F and see table 2.2). Before any opening their density was $198\pm74\#/m^2$, dropping to $1\pm1\#/m^2$ after the opening, whereafter a recovery occurred to $79\pm40\#/m^2$ 2.5 months later (h) (figure 2.2). *Bivalvia* n.i. varied significantly with the interaction of time and space ($p=***$, MS and F and see table 2.2). Before the opening (a), the maximum density was $3\#/m^2$ in station 1 and afterwards in all stations the highest abundance was obtained 1.5 months after the second opening ($12\#/m^2$ in station 3; figure figure 2.2). The density of *N. diversicolor*, *H. ulvae* and ostracoda showed significant differences only between the stations ($p=***$, $p=*$, $p=***$ resp.; MS and F see table 2; figure figure 2.2). *N. diversicolor* had the highest abundance in station 1 closest to the sea ($8\pm3\#/m^2$) whereas in the other stations its abundance stayed low ($< 2\#/m^2$). Ostracoda had the highest abundance ($33\pm20\#/m^2$) in station 5. *H. ulvae* was most abundant in station 1 and 5 ($2\pm1\#/m^2$ and $3\pm2\#/m^2$ resp.).

2.3.2 Spatiotemporal variability of environmental variables

Associated to the artificial opening of the lagoon, salinity varied significantly over time ($p=***$, MS and F and see table 2.2; figure 2.3). Before the opening of the lagoon it was low, on average 1.10 ± 0.1 (figure 2.3). After the inflow of seawater, salinity in the lagoon increased directly to 24.95 ± 7.38 , gradually decreasing afterwards to 17.24 ± 4.59 after five months (h). The acidity differed significantly between the stations ($p=***$, MS and F and see table 2.2). On average station 6 is acid with $pH=6.97\pm0.28$, whereas the other stations have basic pHs ranging from 8.38 ± 0.25 in station 3 and 7.67 ± 0.26 in station 2. Temperature, D.O., Eh and SD varied significantly spatiotemporally (respective p-values, MS and F and see table 2.2). Before the opening of the lagoon the average D.O. was maximum 8.3mg/l in station 1, after the opening oxygen levels increased all over the lagoon (see figure 2.3). Over time the stations in the extreme ends of the lagoon became oxygen depleted, whereas after five months in the middle section (station 4 and 5) the oxygen level reached values similar to the onset of the sampling program.

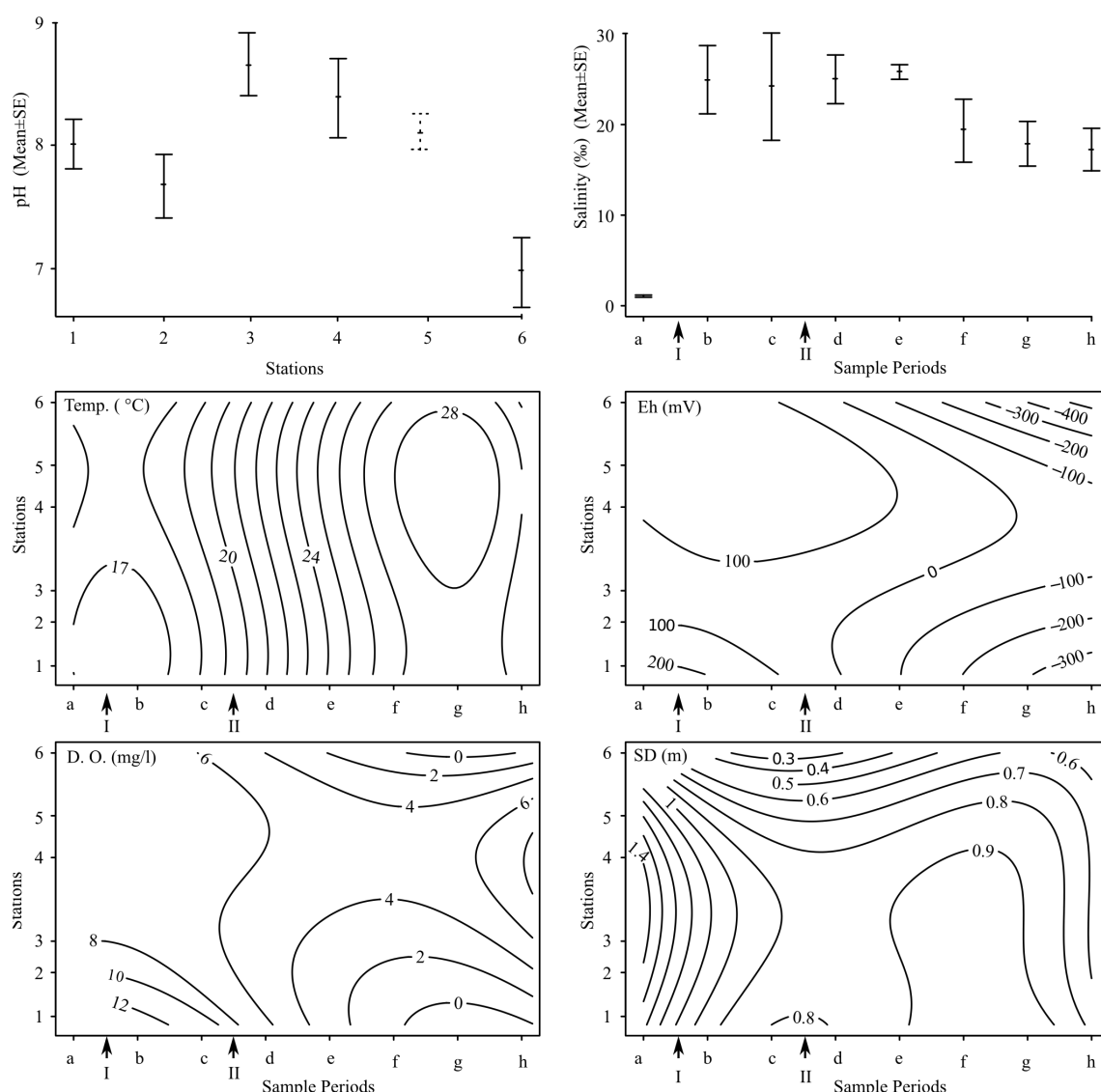


Figure 2.3: Significant results of 2-way anova's (factors 'sample period' and 'station') on environmental parameters: pH in stations (mean \pm SE), salinity (ppt) over sample period (mean \pm SE), and spatiotemporal contour plots of Eh (mV), temperature ($^{\circ}$ C), D.O. (mg/l) and Secchi Depth (SD, m) (p-values, MS and F see table 2.2)

Before opening, average temperature varied around 17° C, with a gradient of 1° C from the front (seaside) to the back of the lagoon, then, the temperature increased during spring and summer to peak to 29° C in the end of the sampling period. In general Eh decreased over the sample period. In the first 3 campaigns, the gradient of Eh over all the stations remained fairly constant with a maximum in station 5 (200.00mV) and a minimum in station 1 (78.50mV). From sample May onwards, Eh manifested higher values in the mid section of the lagoon (39mV and -46mV in station

3-4 resp.) than at both extreme ends of the lagoon (-355mV and -423mV in station 1-5 resp.). Secchi depth was homogeneous throughout the lagoon before its opening. After the opening, the mid section stations maintained a relatively high SD fluctuating around 0.8 m in stations 3-5 whereas in station 1 and station 5 SDs dropped with 0.2m and 0.3m respectively.

Table 2.2: Lagoon status and sampling time

	Factor Time			Factor Station			1-way Granul.		
	MS	F	P	MS	F	p	MS	F	p
Temp	98	99.0	***	3.65	3.89	**	-	-	-
Sal	330	6.32	***	-	-	-	-	-	-
pH	-	-	-	33.37	9.35	***	-	-	-
D.O.	40.76	7.72	***	19.21	3.69		-	-	-
Eh	100159	8.10	***	60212	4.87	**	-	-	-
SD	0.25	7.55	***	0.33	9.88	***	-	-	-
Biv. n.i.	2.90	4.61	***	3.41	5.44	***	-	-	-
<i>H. div</i>	-	-	-	3.99	2.81	*	-	-	-
<i>H. ulvae</i>	1.05	2.39	***	-	-	-	2.19	5.47	**
<i>Chiron.</i>	6.03	1.50	***	-	-	-	-	-	-
<i>L. rugic.</i>	-	-	-	-	-	-	-	-	-
<i>Ostracoda</i>	-	-	-	10.03	6.47	***	-	-	-

2.3.3 Multivariate analysis

D.O. correlated significantly to Eh and Temp. ($r=0.80$ and $r=-0.60$ resp. with $p=***$) and SD to pH ($r=0.43$ with $p=*$). The pca biplot thus only includes salinity, pH and temperature. The first two axes of the pca analysis accounted for 89% of variability (figure 2.4). Salinity and temperature are the two variables structuring most the macrobenthic community. The biplot shows a clear affinity of chironomids with less saline waters, confirmed by a significant relation of their abundance with salinity in the manova ($p=***$, $MS=76.24$, $F=21.36$). *H. ulvae* has the highest abundances with relatively lower temperatures equally confirmed by the manova ($p=*$, $MS=2.74$, $F=7.88$ and $p=**$). It is also the only taxon that varied significantly with the sediment classes characterized by higher abundances in sandy stations (see table 2.2).

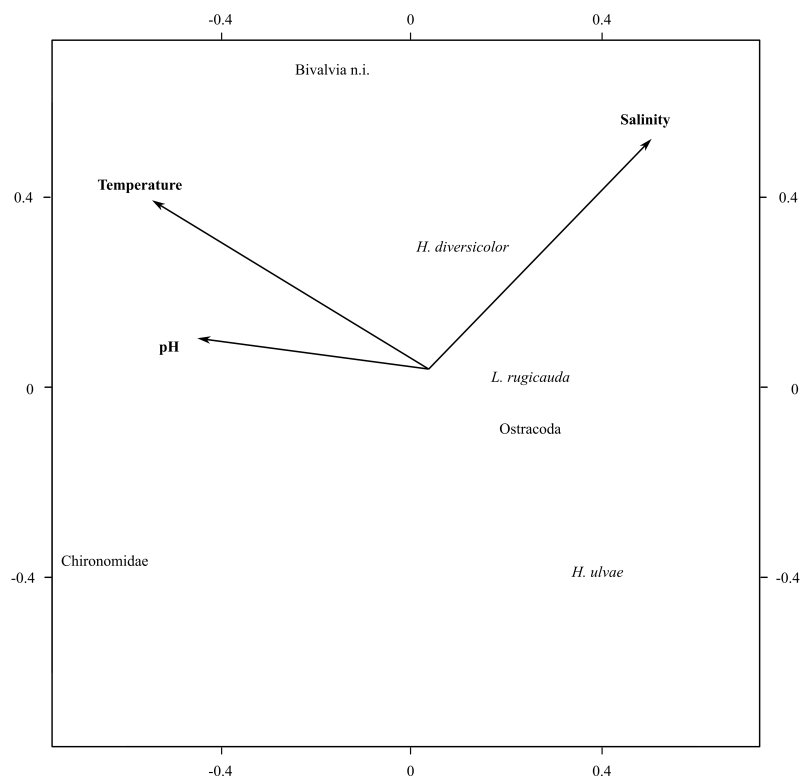


Figure 2.4: Biplot of principal component analysis with arrows representing environmental variables.

2.4 Discussion

2.4.1 Structure of macrobenthic community and ecological status

Features of stressed benthic infaunal communities include presence of r-strategists, high abundances of a few species and low diversity (Elliott and Quintino 2007). *H. ulvae* and chironomids are reported to behave as typical r-strategists, the first in strongly eutrophied areas (Lillebo *et al.*, 1999; Cartier *et al.*, 2011). Even before its opening, it can thus be concluded that the ecological status of the Melides lagoon is poor; confirmed by its extreme low diversity and the dominance by one taxa (chironomids) (Felix *et al.*, 2013).

In other coastal lagoons, salinity was the main environmental factor influencing the benthic community (Blanchet *et al.*, 2005; Pech *et al.*, 2007). For Melides the same was found especially throughout chironomid's affinity for decreasing salinity. *H. ulvae*'s growth rate has been linked

to temperature with lower growth rates during winter (Lillebo *et al.*, 1999), this might relate to the negative correlation of *H. ulvae* with temperature in the present study. For that species food supply measured by algal biomass, has been the most important factor structuring its population (Lillebo *et al.*, 1999; Drake and Arias 1997). Here this might be reflected in the higher *H. ulvae* abundance in a station for which an elevated macro algae cover as previously reported in Antunes *et al.*, (2012).

Impoverished macrobenthic communities comparable to Melides have been observed in lagoons suffering from severe environmental stress by organic over-enrichment of sediments: in Italy for example only 7 of 22 taxa accounted for 94% of total abundance in a eutrophied lagoon (Como and Magni 2009). Low diversity has also been observed in other lagoons in Portugal (Cancela da Fonseca *et al.*, 1999; Carvalho *et al.*, 2005; Drake and Arias 1997). However in the 80'ties a survey in Melides reported 94 taxa (Costa *et al.*, 2003). Although a mitigation of the anthropogenic pressure on Melides was attempted since then (e.g. the construction of a sewage treatment plant and a reduction of urban occupation), its species richness decreased dramatically resulting in a poor ecological status (Felix *et al.*, 2013). Related to this 20 year knowledge gap, it is challenging to assess possible causes of this drastic reduction or pin point when the regime shift occurred. However punctual anthropogenic contaminations have been observed in the hydrogeological system of the Sines basin and were linked to run-off and pesticides from agricultural activities resulting in at times peak concentrations of sulphate and nitrate (Fernandes *et al.*, 2005). Punctual impacts can indeed result in massive mortalities; and combined with continuous physicochemical stress, may maintain the diversity at a low level (Gamito 2006). Water bodies with high residence time, such as Melides, are also characterized by extreme environmental variations over short time frames (Gamito 2008). Degraded water quality combined with enhanced nutrient input may lead to eutrophication in certain parts of the lagoon and ultimately to anoxic events (Afli *et al.*, 2009). The stratification enhanced by temperature, can contribute to the severe bottom-anoxia amplifying the temperature/DO coupling. (Quintino, 1988), Low oxygen values and excessive amounts of decaying algae are recognised symptoms of eutrophication, both of which were observed in Melides (Antunes *et al.*, 2012). The eutrophied status of Melides is also confirmed by the high biomass of chironomids, a taxon used in water quality assessment as an indicator of nutrient enrichment

linked to their resistance to oxygen depletion (Callisto *et al.*, 2002; Viaroli *et al.*, 2008).

2.4.2 Artificial opening as management practice?

The double opening did not result in the colonisation of marine species and only one resident taxon recovered 5 months later. The absence of suitable conditions for recruitment could be an important factor maintaining diversity low in Melides (Felix *et al.*, 2013). Normally an increased marine water influence enhances the number of incoming species and facilitates the establishment of well structured communities in lagoons (Gamito 2006; Tlig-zouari *et al.*, 2009). The number of marine spawning species able to colonize lagoons depends upon the frequency, duration and the timing of the opening is established (Griffiths 1999). Despite the potential for recruitment depending also on stochastic elements (Beer and Joyce 2013), temperature might already provide a straightforward indication. For example *H. diversicolor* first gametes release is induced by a temperature rise (5°C) in early spring and may be limited to a short period (Bartels-Hardege and Zeeck 1990). Melides opening coincided with this spring rise in temperature. It is possible that if Melides had been opened earlier in the year, their spawning, reproduction and thus recovery could have been more successful. The same accounts for *H. ulvae* characterized by at least one reproductive peak in spring (Lillebo *et al.*, 1999). The timing of opening is even more crucial for *L. rugicauda*, as this species reproduces only once a year, in discrete periods initiated by triggers such as lunar cycles (Barnes *et al.*, 1993).

It should also be considered to artificially maintain the lagoon open for longer periods instead of opting for its natural closure. This would allow ample time for both water renewal, resulting in the drainage of the water of the lagoon into the sea, a loss of total organic nitrogen and an increase of total phosphorus (Branco *et al.*, 2007). The Óbidos lagoon is an example of a Portuguese lagoon where practitioners applied this management practice and reported an improvement in water quality (Pereira Coutinho *et al.*, 2012). Furthermore a comparative analysis of the macrobenthic community of Óbidos and Albufeira, revealed that frequent closure of the latter caused water stratification with anoxia forcing a regime shift to an impoverished community similar to Melides (Quintino 1988).

On the other hand the physical environment of the lagoon could be manipulated to a bigger ex-

tent: for example to increase flushing changing the lagoon's boundaries is effective (Elliott *et al.*, 2007). Increased runout and more effective flushing might also be obtained by dredging additional inlets instead of only one main channel while planing the artificial opening to coincide with maximum difference of water levels of the lagoon and the sea (Newton and Mudge 2005). Continuous monitoring of Melides generating a more extensive dataset is essential for the determination and implementation of an effective management plan including other aspects of the lagoon biota, such as their avian fauna and fish community (Griffiths 1999).

Acknowledgements

We are grateful to Fundação para a Ciência e a Tecnologia who funded the Phd candidate (fellowship reference: SFRH/BD/48402/2008). The first author also thanks her colleagues for support in the numerous field trips: P.R. Santos, Pais, M., Henriques, S. Prof. Freitas, M. C and Dr. Cruces, A. of the Geology Department of the Faculdade de Ciências da Universidade de Lisboa are also thanked for kindly providing certain abiotic data and logistic support on the sample campaigns.

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Chapter 3

Measuring trends and signals of
sustainability in oyster population and
production data.

Abstract

Resilience research often includes time series analysis in search of trend shifts. Recently statistical signals including changes in variance and autocorrelation, were proven to be universal indicators of stability. In this paper the suitability of those early warning signals was tested on two case studies: oysters production of France (case study 1) and catch rates of the native oyster in the Solent, UK (case study 2). First, trend analyses were performed and their association to the evolution of the North Atlantic Oscillation (NAO) index was assessed. For the French oyster production, two sinusoidal waves were found in the first and second order residuals with periods of 33 and 8 years. In the Solent, the trend depiction showed after an initial increase, the catch rates of oysters declined over time from West to East. A positive relationship was apparent between NAO and both the production and population data, with a significant correlation in case study 2. Furthermore, a high and significant spectral coherency in the 8 year period of case study 1 revealed a near phase opposition between the influence of NAO and the French oyster production. For the first and second case study, the early warning signals calculated were lag-1 autocorrelation and variance, respectively. In case study 1, the stability of the low frequency wave of the first order residuals could not be assessed, the trend of lag 1 autocorrelation was thus not conclusive. However the high but stable lag-1 autocorrelation of the second residuals, revealed a probable underlying stability. In case study 2 significant increase in variance reflected the instability prior to the decline. It is discussed that in periodic signals the number of residual fluctuations needs to be sufficient to allow the generation of an autocorrelation trend within the limits of the sliding window, used for its calculation. The importance of time scale and appropriate de-trending is also questioned when calculating and interpreting early warning signals as indicators of underlying (in)stability. Finally besides their scientific use, also the utility of early warning signals in managerial settings are discussed.

Keywords

time series, sustainability, resilience, oyster, production, catch rates, early warning signals

3.1 Introduction

The first record of the concept of resilience in the early 1970's, introduced a profound shift in the traditional ecological perspective when Holling (1973) used it to understand non-linear dynamics in predator-prey models. Resilience refers most simply to the ability of a system to recover and is closely related, sometimes confused, with resistance defined in the conceptual model of Elliott *et al.*, (2007) as 'the amount of a given pressure, that can be applied without deterioration of status'. Within an ecosystem gradual changes in underlying drivers usually have little or no apparent impact up to a certain threshold, and then unexpectedly lead to a regime shift (Biggs *et al.*, 2009). In some cases, ecosystems undergoing regime shifts do not return to their original state as inter-related associations enabling the original stable state equilibrium are lost (Adger *et al.*, 2005; Anderssen *et al.*, 2009). At the juncture between two apparently stable states, understanding occurring instabilities might help predict a regime change and therefore possibly allow for active aversion management. *Hysteresis* and bifurcation are associated with the dynamical phenomenon of critical slowing down (CSD) known from non-equilibrium dynamics (Drake and Griffin, 2010). CSD refers to the decreasing rate of recovery from small perturbations to a population's expected trajectory, in the vicinity of a tipping point (Scheffer *et al.*, 2009). One characteristic is that populations close to extinction or in the vicinity of a tipping point, will exhibit observable symptoms associated with CSD (Scheffer, 2010). Rather than looking at the state, CSD can be observed through characteristic changes in the pattern of fluctuations in the system itself (Drake and Griffen, 2010). In less resilient systems, or where a stress threshold has been transgressed, recovery rates slow down and the fluctuations in a stochastic environment, tend to be larger and more time-correlated. Such changes are labelled 'Early Warning Signals' (EWS), indicating the approach of a tipping point. A primary observable change would be an increase in autocorrelation: subsequent states in a time series will become more alike (Scheffer *et al.*, 2009; Scheffer, 2010). By contrast, a stable autocorrelation is characteristic of stationary systems (Box *et al.*, 2008). Other leading indicators that may signal an upcoming transition are the rising variance and skewness of fluctuations (Guttal and Jayaprakash, 2008). Scheffer *et al.*, (2009) suggested the universality of those signals in time series of systems ranging from the brain to financial markets and the climate. Yet few empirical

demonstrations explore the detectability of early warning signals, despite the fact that the approach provides a powerful systemic approach for assessing the risk of regime shift (Biggs *et al.*, 2009). Benthic functional groups such as filter feeders, of which shellfish are an important part, represent an opportunity to evaluate the appropriateness of the EWS methods. When nutrients are in excess e.g. during a phytoplankton bloom, shellfish provide an ecological buffer by changing material processes and transferring nutrients from the water column to benthic sediments in bio-deposits (Newell, 2004). Another ecological role of bivalve reefs in estuarine systems is the addition of physical structure serving as anchoring for organisms and increasing local diversity patterns. The species evaluated here are Native (*Ostrea edulis*) and Pacific Oyster (*Crassostrea gigas*), keystone species which are part of the Biodiversity Action Plan (BAP). Shellfish are not only systemic buffers, they also represent a historically important food source for society as well as a source of income to fishermen and ancillary services. The World Health Organisation (WHO) rates the contribution of bivalves to the total global trade in fish and fishery products around 2.3%, but stresses its high regional importance (WHO, 2010). Considering the economic, nutritional and ecological interest of bivalve molluscs, it is worthwhile to explore population and harvesting time series in the following aspects: trends, with a focus on possible periodic components, as well as testing whether EWS can be observed.

Natural climatic cycles are known to influence bivalve mortality through their effect on environmental variables, such as water temperature and salinity (Soniati *et al.*, 2009). A harmonic trend with periodicity of 4 years, corresponding to El Niño/La Niña-Southern Oscillation (ENSO) was found when correlating Dermo-disease infection in *Crassostrea virginica* with abiotic parameters (Soniati *et al.*, 2009). Moreover, along the Eastern Atlantic coast of the USA, the primary correlation between temperature and oyster mortality, with a periodicity of 8 years, was associated to positive North Atlantic Oscillation indices (Soniati *et al.*, 2006). The decline of the recruitment success in cockles and other bivalves in the Wadden Sea was linked to climate change (Beukema and Dekker, 2005). The frequency analyses of phases of phenomena such as ENSO and NAO is thus worthwhile as bivalves species do respond to climate induced variations in their environment. Cross-spectral analyses could also be used as this method is applied in many fields when assessing the cross-correlation between two periodic time series: for example, used when examining

spatiotemporal drought variability (Touchan *et al.*, 2011).

This paper uses two case studies, productivity and population data of oysters, both characterized by significant fluctuations, and located in regional proximity. The first objective is to identify and visualise their trends both time and space-wise and examine possible influence of NAO. Finally appropriate EWS for two case studies will be identified and their potential as resilience assessment tool reflecting the underlying (in)stability will be assessed.

3.2 Material and Methods

3.2.1 Case studies

Two time series of annual oyster fisheries data were interrogated. Case study 1 is the oyster production of France from 1950 to 2008 and case study 2 investigates the scientific survey catch rates of the native *Ostrea edulis* in the Solent (UK) over 37 year (1973–1980 and 1990–2009). The data for the French oyster production, in tonnes of live weight, were obtained from FAO statistics (2010). France is presently one of the leading countries in Europe for shellfish production (Goulletquer and Le Moine, 2002). Oysters are harvested both on its Mediterranean coasts and Atlantic coasts although most of the ten regions known as oyster producing areas are situated on the Atlantic coast and the English Channel (figure 3.1a). Marennes-Oléron represents the largest oyster cultivation area accounting for 45% of French oyster production (Goulletquer and Le Moine, 2002). The Mediterranean sites are situated near the Gulf of Lion (Buestel *et al.*, 2009).

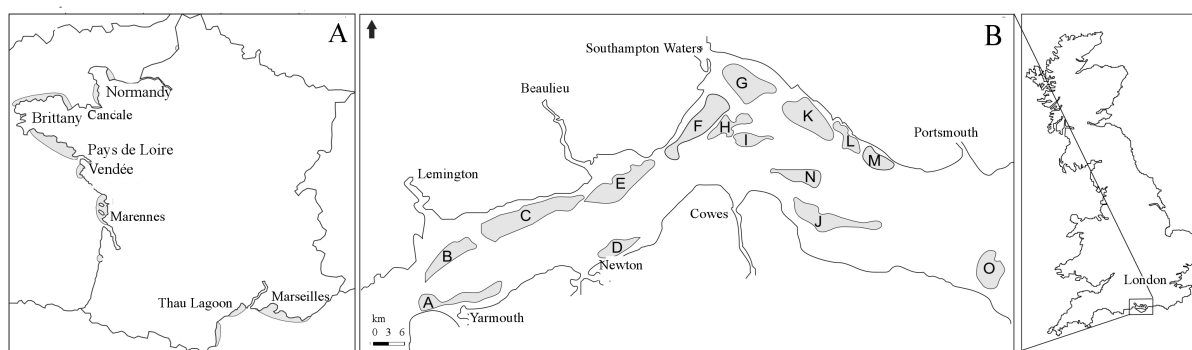


Figure 3.1: A. Case study 1: National oyster production of France: Location of the major harvesting areas around Atlantic coast and Mediterranean coast. B. Case study 2: Oyster catch rates of the Solent: Surveyed oyster grounds. (abbreviations: see Material and methods).

For the Solent, catch indices were compiled from technical reports from an ongoing annual monitoring programme carried out by Cefas, a UK government research institution (Key and Davidson, 1981; Vanstaen and Palmer, 2009). The Solent is an estuarine complex located on the central South coast of England. The area consists of 12 separately defined estuaries and harbours, including those on the Isle of Wight (figure 3.1b). There are a multitude of competing demands on, and uses of the area: industrial, recreational, fishing, shipping and social (Fletcher *et al.*, 2007). It is a low energy, generally shallow macro-tidal system with a mean tidal range of 3.2 m. There is a marked increase in range from west to east, and the local topographic configuration interacts with the phase of the twice a day natural oscillation to generate a double high water in the West Solent and Southampton Water (Iriarte and Purdie, 2004). Because of the progressive nature of the tidal wave the long term residual transport in the Solent is towards the west and the flushing time is long (Dyer and King, 1975). The area encompasses large native oyster beds and the Solent's oyster fishery was historically the largest naturally recruiting *Ostrea* population in Europe (Tubbs, 1999). Data on 14 monitored oyster beds are used in this analysis: A: Yarmouth Road, B: Lymington Banks, C: Sowley Ground, D: Newtown Bank, E: Lepe Middle Shoal, F: Stanswood Calshot, G: Chiling Ground, H: Thornkoll, I: Bramble Bank, J: Mother Bank and Osborn Bay, K: North Chanel, L: Lee-on-Solent, M: Browndown, N: Ryde Middle Bank, and finally O: Warner Shoal (figure 3.1b). Two size classes of oysters were considered: size class I (< 49 mm) and size class II (> 49 mm) to be able to distinguish effects on the juvenile or adult population. Catch data was obtained using a Baird oyster dredge fitted with a 91 cm blade and rigged with a 40 mm square mesh chain-link bag, towed for approximately 100 m at each oyster bed on a defined grid (Vanstaen and Palmer, 2009). Number and size category are recorded per haul per oyster bed.

3.2.2 Statistical analyses

For all analyses .R software, version 2.10.1 is used (.R Development Core Team., 2009). The approach for the trend analysis and EWS calculation differed for both case studies. A first empiric analysis of the French landings revealed clear cyclic trends and was further examined with Fourier

transforms whereas for the Solent oysters, Cefas's reports already highlighted their decline, so the analysis focussed on a spatiotemporal trend visualisation.

Cyclicality in case study 1

Cyclic trends were tested by performing a spectral analysis using fast Fourier transforms (FFT). To improve the statistical properties and to prevent leakage, the time series was padded and tapered for 10% as recommended by Bloomfield (2000). A linear model was fitted using generalised least squares to remove linear trends and its first order residuals with zero mean were plotted. Second order residuals of the initial time series were also calculated: using a curve-fitting approach a low frequency sinusoid identified in the FFT of the first residual was extracted from the 1st order residuals (Bloomfield, 2000). The FFT was run also on the second order residuals and both FFTs were visualised by their spectral density-periodogram.

Decline in case study 2

To allow same scale cross-comparison of catch indices from different oyster beds, data is normalised. The normalisation is done by calculating the oyster catch for each oyster bed relative to each bed's total abundance over the time period (%). This technique, focuses on the dynamics of catch indices rather than absolute catch differences between the grounds as reported in Vanstaen and Palmer (2009). The ratio of small and large oysters versus total population before and after the decline was tested for its significance using a Welch Two Sample t-test. To visualise the common trend in the oyster beds, data was aligned so that the maximum catch rates of beds coincided. Finally, to express the spatiotemporal trend, a principal component analysis was run using the oyster beds as variables and the normalised catch rates per year as the observations. A pca biplot is presented with annual catch rates as scores and the biplot arrows indicating the oyster beds.

3.2.3 EWS reflecting (in)stability

As case study 1 spans a larger time period than case study 2, different approaches were necessary to assess EWS for both case studies: lag-1 autocorrelation was used for case study 1 whereas variance was calculated for case study 2. In the latter, only the data ranging from 1990 to 2009 was used as it was the longest continuous time series. The autocorrelation was calculated as in Dakos *et al.* (2008) where a time-window consisting of half the length of the time series is used. The window was then automatically slid over the time series. Lag-1 autocorrelation was calculated for the first and the second residuals smoothed using the Nadaraya–Watson kernel regression with $bw = 3$. The ‘acf’ function of the .R ts-package is based on the formula at lag 1 (ρ) (Pinheiro and Bates, 2000) in 3.1:

$$\bar{\rho}(l) = \frac{\sum_{j=1}^{N-l} r_j r_{j+1} / N(l)}{\sum_{j=1}^N r_j^2 / N(0)} \quad (3.1)$$

with:

l: the lag

r_j = the residuals from the generalised least squares approach

j= the group residual pairs within the selected lag

$N(l)$: the number of residual pairs within the selected lag

$N(0)$: the total number of residual pair

In case study 2 the variance of the yearly catch rates prior to the decline was calculated as follows in 3.2:

$$s^2 = \frac{\sum_{i=1}^N (x_i - \bar{x})^2}{N - 1} \quad (3.2)$$

N: the total number of observations

x_i : actual value

\bar{x} : average of time series

The same formula was used to assess the variance of the size class I of oysters before their respective declines. In both cases the variance was calculated on the logarithms of the catch rates to avoid scale bias.

3.2.4 North Atlantic Oscillation

The indices of the yearly average North Atlantic Oscillation from 1950 to 2010 were downloaded from the NOAA site. Both its longer trend (low frequency) and its shorter term variations were compared with the oyster population and production data. For case study two, a Pearson correlation was performed between NAO indices and the general trend of oyster catch rates. In this case the total oyster catch per year of the Solent over the period 1990–2009 was compared to the evolution of the NAO over the same time period. To further compare the low frequency behaviour, the following three variables we plotted: average NAO/year (smoothed, $bw = 15$), the range of average Solent catch per year (smoothed, $bw = 15$), and the low frequency sinusoid of French oyster production. The first 2 variables were smoothed with $bw = 15$ as to eliminate any higher than decadal trends. Like-wise, shorter term periodicities of French Landings and NAO were compared. A cross spectrum with smoothing spans of 3, has been performed on the French Oyster second order residuals and the NAO residuals from its $bw = 15$ trend (Bloomfield, 2000). The original data has been normalised to their respective range. The cross spectrum is visualised by the squared coherency with a 95% confidence interval and the values of the phase difference at maximum coherence.

3.3 Results

3.3.1 Cyclic trends in case study 1

Over a 60 year period the French oyster production varied widely (figure 3.2a). Production fluctuated from around 80–120 thousand tonnes during the 1950's. In 1971 a drastic decline of more than 26 thousand tonnes occurred with respect of previous year. However a gradual increase in production took place in the 70'ties through the 80'ties to reach high levels in the 90'ties with a maximum of 152 thousand tonnes oysters landed in 1996.

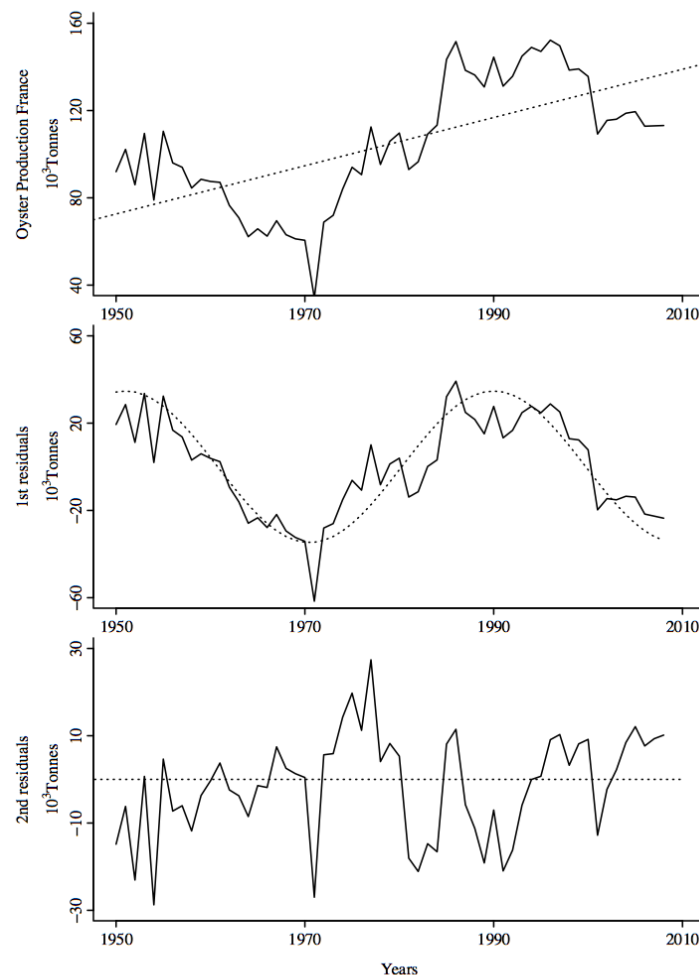


Figure 3.2: a. Productivity statistics of French oyster production: Landings (tonnes live weight) from 1950 to 2009. b. First residuals of French oyster production. c. Second residuals of French oyster production.

The first residuals reveal what might appear to be a long period harmonic trend, as the raw data, but its linear increase was removed (figure 3.2b).

Its spectrogram showed indeed a dominant sinusoid with frequency 0.03, corresponding to a period of 33 years (figure 3.3). De trending from this low frequency sine wave, a cyclicity seems to persists in the second order residuals (figure 3.2c). This is confirmed in its spectrogram where the 0.12 frequency, corresponding to a period of 8 years, singled out particularly strongly (figure 3.2b).

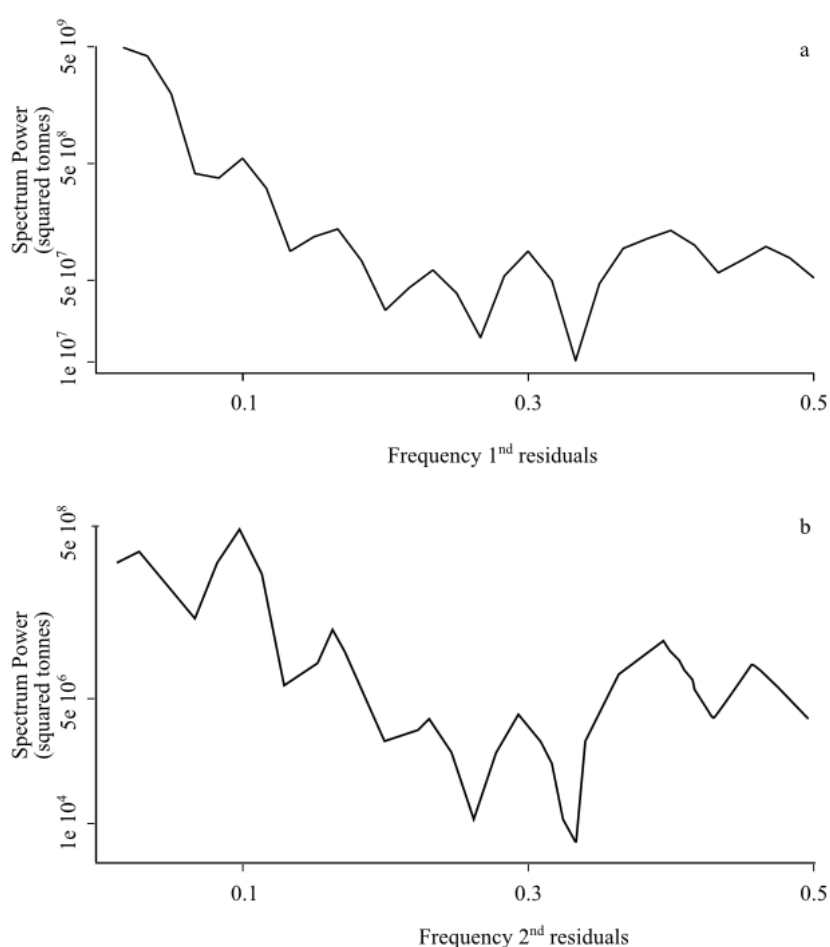


Figure 3.3: Frequency spectrogram of the first and second order residuals of the French oyster production data

to East decline: as from 1973, the following years were plotted clockwise following the west-east gradient of the oyster beds (figure 3.4 a). The first two axes of the pca analysis explained 68% of the variability in catch rates of the different oyster beds over time (figure 3.4 b). The beds located in the West were characterized by higher relative catches in the early years, whereas the beds situated in the East had clearly higher catches from 2000 onwards. The ratio of small and large oysters versus total population changed significantly before and after the notable declines: the oysters were significantly larger after the decline ($t = -2.78$; $df = 12$; $p < 0.01$).

3.3.3 North Atlantic Oscillation

From the 60'ties the low frequency pattern of the French landings is nearly identical to the main trend variation in average NAO indices (figure 3.5 a). Likewise, the decline in the total Solent catch rates from 1990 to 2010 coincides with the downward trend in average NAO. At 0.12 frequency, (8 years), the cross-spectrum shows a significant coherency of 0.8 between NAO and the French Oyster residuals (range 0.35–0.95) (figure 3.5b). At this peak, both time series are out of phase, by $-2.5 (\pm 0.5)$ radians, close to phase opposition. A significant Pearson correlation confirmed the correlation ($p = 0.02$) of the increase in total Solent catch rates with higher NAO indices for the period 1990–2009 (figure 3.5c)

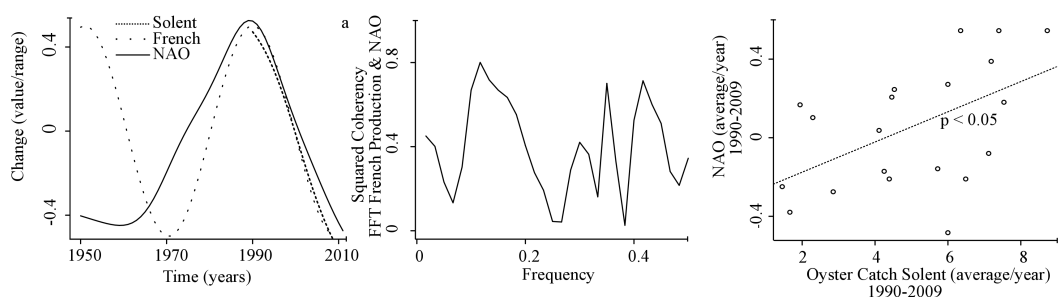


Figure 3.5: a. Change of average NAO/year, average Solent catch per year, and the low frequency sinusoid of French oyster production from 1950 to 2010. b. Frequencies of the FFT of French production and NAO with their respective squared coherency and 95% confidence interval. c. Total oyster catch in the Solent and the NAO indices over the period 1990–2009 with the significant Pearson correlation ($p < 0.05$)

3.3.4 EWS reflecting (in)stability

The autocorrelation of the first residuals of the french landings showed a clear variation which is consistent with the potentially cyclic long term trend, between a maximum of 0.81 and a minimum of 0.42 (figure 3.6a). The autocorrelation of the second order residuals showed a stable lag -1 ACF coefficient fluctuating between 0.91 and 0.70 as respective minimum and maximum (figure 3.6b). The variance of the total Solent oyster catch increased significantly before the decline ($r = 0.5$ $p < 0.05$) (figure 3.6 c). The increase in variance is even more accentuated for the oysters smaller than < 49 mm ($r = 0.9$, $p > 0.001$) (figure 3.6d).

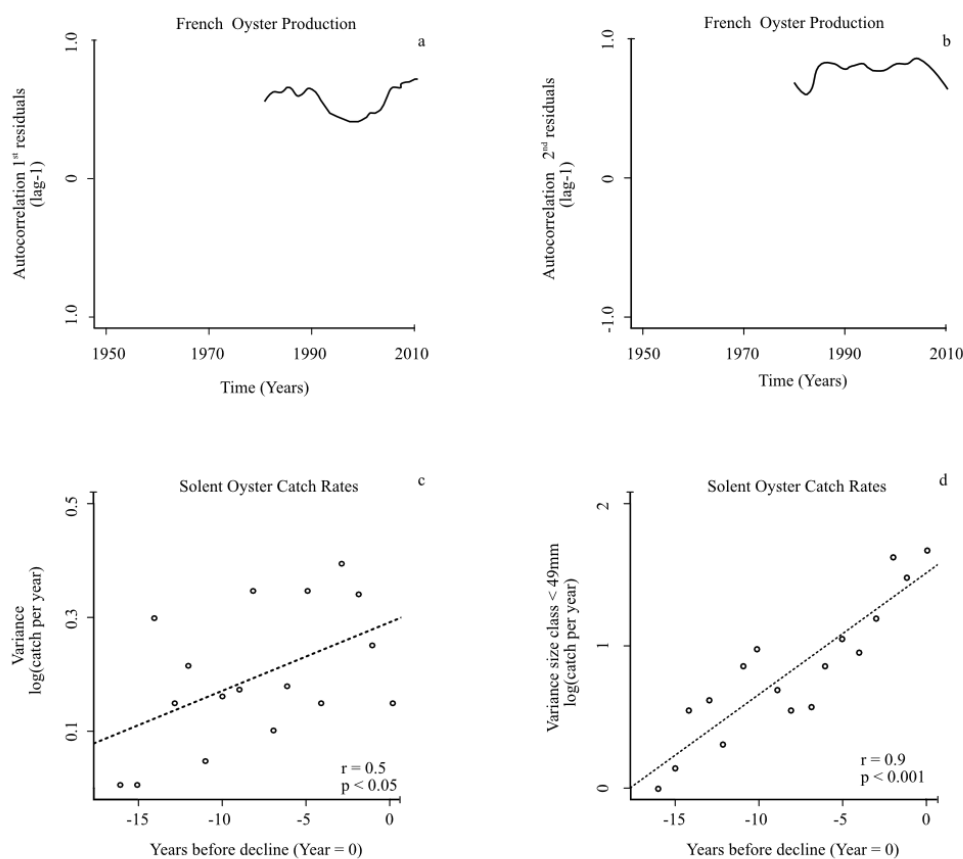


Figure 3.6: Early Warning Signals for two case studies. a. Lag -1 autocorrelations for the first residuals of the French oyster production data. b. Lag -1 autocorrelations for second order residuals the French oyster production data. c. Variance of total catch rates of oyster beds before their respective declines in the Solent. d. Variance of catch rates of size class I (< 49 mm) before their respective declines in the Solent.

3.4 Discussion

3.4.1 Data collection

In the French production case study, landings are conveniently used as publicly available indicators related to the stock state and production. The quality and accuracy of the official fishery landings statistics may be questioned considering reporting consistency (Kleiven *et al.*, 2012). Improvement of the system of data collection is indeed an issue which FAO is addressing in different ways (FAO, 2013). For example, for quality assurance, FAO's data collection methods and landings declarations have been using customised national questionnaires (FAO, 2010). This analysis, focuses on the evaluation of trend methodologies, the absolute accuracy of the landings is not of major importance, if the trends are sufficiently unbiased (Buestel *et al.*, 2009). In the Solent native oyster survey, historical catch rates are used as a proxy of oyster abundance and form the basis for scientific advice on fisheries management, commonly used in fishery assessment (Maunder and Punt, 2004).

For the analysis, both cases were selected to provide from a methodology perspective, different approaches to trend and fluctuation analysis within related species. The case studies are also regionally close and were coherent in their relationship with NAO. Likewise, EWS as a lack of sustainability was identified in the system which declined to collapse, the Solent, and not in the system which appears sustainable, in spite of its considerable cyclicity.

3.4.2 Cyclic trends in case study 1

Temporal trends in oyster mortality along the coast of France have already been investigated in Soletchnik *et al.* (2007) with similar methodology to the present study. However, the time frame investigated was too narrow to detect the two harmonics with periods of 33 and 8 years found here. As the productivity statistics include aquaculture, disease causing mortalities and declines in landings have been reported, likely exacerbated by extreme densities in ponds: for example in 1971, a major viral infection slashed the production of *C. angulata*, which was then replaced in 1972 by *C. gigas*. (Buestel *et al.*, 2009). In this case it was human intervention and management

decision that upon the downfall of the cycle, got the production up again and made the long term cyclic trend possible. Similarly the drastic drop in oysters (*O. edulis* and *C. angulata*) observed in the late 1960's and early 1970's along the north west coast of Brittany and rapidly spreading to the rest of France, were linked to multiple oyster diseases, a crucial one being the parasite *Marteilia refringens* (Alderman, 1979).

Correlation between natural climatic cycles and cyclic trends in bivalve disease infection have already been demonstrated (Soniat *et al.*, 2009). In the Delaware Bay (USA), the primary correlation between temperature and oyster mortality, with a periodicity of 8 years, was associated to positive North Atlantic Oscillation indices (Soniat *et al.*, 2006). In the French case study, a significant spectral coherence of the sinusoidal modulation of the oyster production with periodicity of 8 years and the periodicity of the average NAO indices confirmed this. At low frequency (33 years) their trends were similar, but further coherency could not be confirmed as the time series were too short. Caution is needed with the interpretation of the NAO influence as national declines might mask regional differences or trends. While replicates are not possible within the same time frame and region, the present study provides encouragement for extended analysis to specified production data from each region and test how this periodicity compares from region to region. In addition to biological phenomena, the production figures also reflect consumer's market preferences as an anthropogenic component. When shellfish farming is analysed through a demand-oriented-approach, it is clear that higher demands result in improvement of techniques or higher harvest and consequently higher production numbers (Girard and Mariojouis, 2003).

3.4.3 Decline trend in case study 2

The normalisation of the data proved to be particularly useful to visualise the dynamics in oyster catch rates over time. The shape of the normalised catch rates together with the pca diagram revealed a depletion occurring on one bed after the other from West to East, with the exception of the most western bed. However, due to its peculiar spatiotemporal pattern it is difficult to assess the decline. The lack of measurements of environmental variables specific to the surveys does not facilitate the interpretation as a tight link exists between the environment and the recruitment and survival of larvae, the most sensitive life-stage of invertebrates and crucial for stock sustainability

(Taris *et al.*, 2006). The NAO indices showing an influence on the total oyster catch rates with negative NAO associated with lower catch rates, is too global to account for the local pattern of the decline. What certainly contributed to the diminished recruitment is the altered sex ratio's confirmed in the Solent, with an unusual high proportion of males in the population (Kamphausen *et al.*, 2011). After the decline significantly more older individuals were caught reflecting the age structure of the population moved to older individuals. Furthermore, the current low densities might be even more negatively impacted by the Allee effect whereby at low densities individuals are too far apart to successfully reproduce (Drake and Griffin, 2010). Finally the decline probably has multi-factorial causes, the interaction of which gave rise to the particular curve and geographic pattern in the declines over time.

3.4.4 EWS reflecting (in)stability ?

Early warning signals as proposed by Scheffer *et al.* (2009) belong to a family of generic leading indicators that may help to determine whether a complex system is on the brink of collapse. Their relationship with resilience of systems can be summarised as follows: systems with low resilience are characterized by a large variability and events are more time correlated, whereas highly resilient systems have a deep basin of attraction, allowing rapid return to equilibrium after a fluctuation, less autocorrelation and a lower standard deviation (Scheffer, 2010). Drake and Griffen (2010) found experimentally that the same plankton system can have stationary fluctuations, cycles or chaos-like dynamics under different environmental conditions. Furthermore, the temporal scale is crucial as different perspectives on the resilience might be perceived depending on time frame analysed. In the present study, this argument is reinforced where snapshot analysis might lead to erratic conclusions of declines, where in fact stable cyclic trends are characteristic of the production statistics. Particularly important is the size of the window, used for the calculation of the autocorrelation, in comparison with the periodicity of the fluctuations. A long term trend might be fluctuating in a sustainable way but if not stationary within the time frame analysed, ACF will be influenced (Dakos *et al.*, 2012; Lenton *et al.*, 2012). In this paper, the low frequency harmonic in the French case study is a long term trend of which the stability cannot be proven, as the available time scale is only a bit longer than one period. The lag-1 autocorrelation cyclicity associated to it, is thus

inconclusive. When de-trending the data from this low frequency sine wave, the short term fluctuations (8 years) indicated a stable system with high, but not further increasing autocorrelation of the second residuals. In shorter time series variance as early warning signal proved to be effective as shown by the second case study where it effectively picked up the increased variability prior to the decline in oyster catch. One other critical issue for effective EWS resides in the frequency of the measurement: supplementing abundances of species with long life cycles, with measures reflecting altered primary production or nutrient recycling might provide sufficiently early signals of stress linked to the shorter turnover.

Despite the application of EWS being rare in managerial settings, early-detection methods are assessed by different criteria including their applicability to real ecosystem monitoring and management (Lindeberg *et al.*, 2012). Accurate management of marine resources asks for timely EWS with a straightforward calculation and interpretation as to facilitate decision making. Recently online toolboxes, flowcharts and literature on their robustness and sensitivity provide valuable guidelines for computing autocorrelation and variance (Dakos *et al.*, 2012; Lenton *et al.*, 2012; <http://www.early-warning-signals.org/>). However both autocorrelation and variance as EWS inherently require lengthy time series and in commercially exploited oyster populations, the spatiotemporal quality and consistency of the monitoring program will influence EWS. For example in contrast to a yearly sampling, monthly measurements are more likely to reveal upcoming non-linear changes quicker. In the later case, if need-be, seasonality can be factored-out, for example, with twelve-month moving average, in monitoring plankton changes (Reid *et al.*, 1998). For naturally recruiting bivalve stocks, besides directly performing EWS on oyster parameters/catch rates as in this paper, a previously identified suitable indicator of overall ecosystem could also been subject to EWS calculation. For example two copepods, key indicator species, contributed significantly to a regime shift in the Baltic Sea and proved effective EWS candidates for the assessment of the ecosystem health (Lindeberg *et al.*, 2012). A hurdle for the use of EWS in management is how far ahead of the regime shift the warning may actually be picked up. However by applying multiple EWS, this can be partially overcome: for example variance and autocorrelation changes appeared a maximum 2 years in advance, whereas alternative approaches such as shiftograms assessing structural breakpoint by combining several statistical indicators of time series, seemed a promising tool

to an earlier detection (Lindeberg *et al.*, 2012).

3.5 Conclusions

While cycles in the French case indicate re-bound capacity of oyster production, including favourable human interventions such as the introduction of alternate species, the Solent case highlights the West East progressive decrease in oyster beds. In both cases early warning signals have proven to be useful tools to assess potential underlying (in)stability: in case study 1, the autocorrelation of the second residuals revealed a probable underlying stability whereas in case study 2, the variance increase, constituted a significant EWS. Cyclicity is an important factor potentially affecting the analysis of any EWS and their spectral analysis constitutes a powerful supplement. It identifies stable periodicities, provides opportunities to associate them with external cyclic factors and designates them as potential de-trending candidates for further residual analysis. Yet, the number of residual fluctuations needs to be sufficient to generate a meaningful autocorrelation trend within the limits of the sliding window used for its calculation.

Acknowledgements

The first author was funded by a PhD fellowship of Fundação para a Ciência e a Tecnologia with reference ateful to Fundação para a Ciência e a Tecnologia who funded the Phd candidate (fellowship reference: SFRH/BD/48402/2008). The authors would like especially thanks the Cefas team for its continuous constructive input.

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Chapter 4

Evidencing a regime shift in the North
Sea using early warning signals as
indicators of critical transitions.

Abstract

One of the longest marine monitoring programs in the North Sea has been the spatiotemporal surveying of subsurface plankton since 1931. During this period a regime shift was detected in the late 1980s culminating in marked changes in phytoplankton, zooplankton and in the fisheries of horse mackerel. Here we used the phytoplankton colour index, a visual biomass estimate, from 1948 to 2010 and total diatom abundance from 1958 to 2010 to test whether the well-documented regime shift could have been anticipated by the recently developed Early Warning Signals for critical transitions (EWS). We estimated EWS, namely autocorrelation and standard deviation, within moving windows along the time series prior to the regime shift. We found that both statistics increased revealing that the North Sea ecosystem was becoming progressively unstable prior to the regime shift. Moreover, this high-resolution time series permitted us to test for robustness, error and significance of the EWS. We did that by dividing the time series into independent blocks and estimating EWS after bootstrapping and randomising the blocks. This alternative approach confirmed the robustness of the EWS with limited associated errors. In particular, we found that the warning was significantly evident years before the onset of the regime shift. We conclude that EWS may provide robust and timely warning for upcoming regime shifts depending on the quality and quantity of recorded data in marine ecosystems.

Keywords

resilience, North Sea, phytoplankton, early warning signals, regime shift, diatom

4.1 Introduction

Ecosystems at times don't respond gradually to changing external pressures but undergo abrupt changes, regime shifts detected in terrestrial, freshwater and marine ecosystems (Andersen *et al.*, 2009; Mollmann *et al.*, 2011). Regime shifts are driven by gradual external environmental changes like overexploitation of fish stocks or eutrophication, and they are triggered by stochastic perturbations such as climatic fluctuations or strong hydrological disturbances (Andersen *et al.*, 2009; Scheffer *et al.*, 2001). In the North Sea, such a regime shift was detected in the late 1980s characterized by extensive changes on three trophic levels (Reid *et al.*, 2001; Weijerman *et al.*, 2005). At that time, phytoplankton stock monitored by the 'phytoplankton colour index' (PCI), which correlates well with fluorometric and satellite derived chlorophyll-a concentrations, increased significantly (Batten *et al.*, 2003a,b; Leterme *et al.*, 2005). Simultaneously zooplankton species abundances changed and the catches of the horse mackerel experienced a step-wise increase of a 4-fold increase over 5 years (100×10^3 to 400×10^3 tonnes) (Reid *et al.*, 1998a,b; Reid and Edwards 2001). Due to the fact that regime shifts might have socio-economic implications as they alter ecosystem's goods and services, the development of tools to anticipate, and thus, potentially mitigate them is crucial for the management of biotic resources (Boettiger *et al.*, 2013; Dakos and Hastings 2013, Elliott, *et al.*, 2007). One recently suggested method for the detection of regime shifts uses early warning signals (EWS) (Scheffer *et al.*, 2009; Scheffer *et al.*, 2012; Drake and Griffen, 2010; Lenton 2011; Wang *et al.*, 2012). EWS are statistical indicators that measure the dynamic phenomenon of 'critical slowing down' (CSD). CSD is defined as decreased recovery rate when ecosystem's resilience is low (Scheffer 2010). As CSD occurs close to a transition, ecosystem variables tend to become more temporally correlated and show a higher variability. This results that time series of stable ecosystems (far from a transition) have distinct correlation and variance patterns when compared to less stable ecosystems (close to a transition) (figure 4.1a,b) (Dakos *et al.*, 2012a). In that sense, autocorrelation and standard deviation are both theoretically expected to increase in case of loss of resilience as ecosystems approach a transition and can be used as EWS (Scheffer *et al.*, 2009; figure 4.1c).

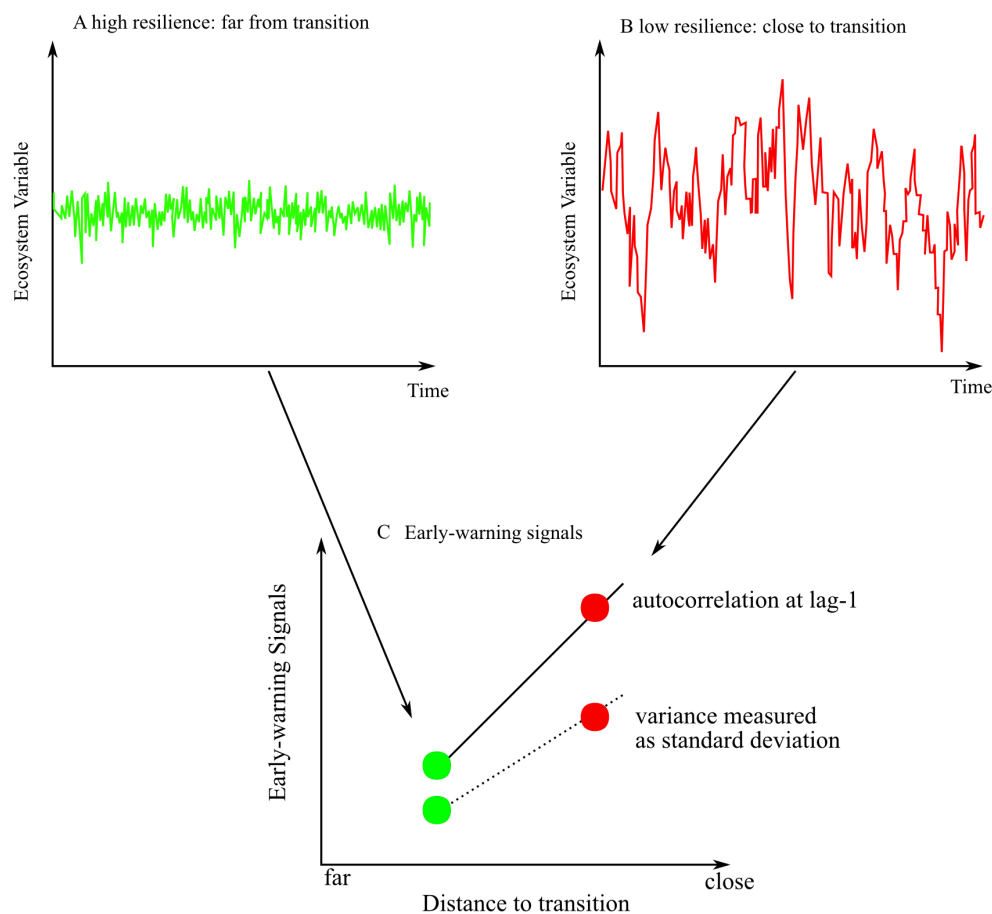


Figure 4.1: Conceptual diagram of ecosystem dynamics far and close to a critical transition. a. hypothetical time series of an ecosystem that is far from a transition (high resilience). b. hypothetical time series of the same ecosystem but close to a transition (low resilience). c. Autocorrelation at lag-1 and variance (measured as standard deviation) both increase when the ecosystem is close to the transition (b) due to critical slowing down and can therefore be used as Early Warning Signals (EWS) indicating the loss of resilience and the proximity to a transition.

EWS have been demonstrated to occur prior to paleo-climate transitions (Dakos *et al.*, 2008), lake eutrophication (Wang *et al.*, 2012), trophic cascades (Carpenter *et al.*, 2012), experimental zooplankton extinctions (Drake and Griffen, 2010), or the collapse of photo-inhibited green algae (Veraart *et al.*, 2012).

Despite EWS's promising applications, care is needed in the interpretation of the results, as not all regime shifts are associated with CSD (Dakos *et al.*, 2012b; Lenton *et al.*, 2012a,b; Boettiger *et al.*, 2013). Moreover, from a practical perspective, testing these tools in the field is limited due to the high quality time series required (Boettiger *et al.*, 2013). Robust detection of EWS depends on the frequency of the observations (e.g. yearly versus monthly), and the turn-over of

the variables upon which they are applied: measures of primary production or nutrient recycling might be more appropriate than species abundance with long life cycles (Wouters *et al.*, 2013). Indeed, comparing multiple EWS leads at times to discrepancies that are not always in line with theoretical expectations (Dakos *et al.*, 2012b; Lindegren *et al.*, 2012). One main constraint in the use of temporal indicators relates to the length of the time series: shorter time series may limit robustly detecting EWS as a lengthy timespan prior to the regime shift is necessary (Dakos *et al.*, 2012 a,b).

Despite the well-developed theoretical background for EWS, the above limitations have restricted real world testing especially in marine ecosystems. Few studies have addressed their potential of detecting marine regime shifts so far. Lindegren *et al.* (2012) estimated EWS in a large-scale reorganization of the Baltic Sea ecosystem using two indicator copepod species but found no clear patterns. Litzow *et al.* (2008) used a measure of community composition of trawl survey data and observed increased spatial variability accompanying the reorganisation of two continental shelf ecosystems in the North Pacific (Gulf of Alaska) and the North Atlantic (Scotian Shelf), while Litzow *et al.* (2013) compared collapsing and non-collapsing fisheries models of Crustacean fisheries. Despite this work, we still lack understanding on the applicability of EWS as tools for anticipating regime shifts in marine ecosystems.

In this study, we tested the validity of EWS in anticipating a marine ecosystem regime shift in retrospect. We chose the well-established North Sea regime shift to assess whether and how early it could have been anticipated using EWS. Our overall aim was to evaluate whether the particular marine regime shift could be considered a critical transition characterized by CSD. In addition, our results indicate whether EWS have the potential to forecast upcoming regime shifts in marine ecosystems. To this end, we develop a new method for applying and interpreting EWS in a marine context that can be of use in cases of available long-term and high-resolution data sets.

4.2 Material and methods

4.2.1 Case study: regime shift in the North Sea

We used monthly data of phytoplankton biomass from 1948 to 2010 based on a visual estimate given by the ‘phytoplankton colour index’ (PCI) and total diatom abundance data from 1958 to 2010 (Edwards 2013). Samples were obtained with the Continuous Plankton Recorder (CPR),

which is one of the longest running and spatially extensive marine biological surveys in the world (Reid *et al.*, 2001). Basically the coloration produced by chlorophyll pigments of silk bands of 10 nautical mile samples is gauged in the laboratory with reference to standard colour charts based on a four rank-order categories (Reid *et al.*, 2003; Batten *et al.*, 2003a,b; Leterme *et al.*, 2005). This ordinal scale is re-assigned to numerical values to best fit actual colour intensity. Under high magnification (x450), diatoms are counted in 20 fields of view (diameter 270 μm) across each sample (a detailed description of the procedure can be found in Richardson *et al.*, 2006). Based on the PCI index a regime shift in the North Sea around 1988 has been suggested (Reid *et al.*, 1998b).

4.2.2 Data processing

For the monthly PCI values, the CPR data of standards areas C1 and C2 (central North Sea) were pooled into a joint mean by dividing the total PCI values of C1 and C2 of the given month by the total number of samples in both areas (figure 4.2a). Diatom abundances of C1 were used and log-transformed. In further analyses, the data were normalised to range and centred to mid-range. We eliminated seasonal trends in PCI by calculating the anomaly as follows:

$$\text{PCI Anomaly} = \frac{PCI_{(\text{ObservedYear}),(\text{CalendarMonth})}}{\sum PCI_{(\text{CalendarMonth}),y} / (\text{ObservedYear} - 1948)} \quad (4.1)$$

As a tool to visualise the regime shift and summarise major changes in the time series of both variables, we used cumulative sum (cusum) of deviations from the time series mean (Andersen *et al.*, 2009). Successive negative residuals below the mean will result in a negative slope and successive positive residuals above the mean will result in a positive slope (Edwards *et al.*, 2006).

4.2.3 Early Warning Signals (EWS)

We estimated autocorrelation at lag-1 and standard deviation on the normalised and seasonally detrended time series of both PCI and diatom abundances. Both EWS were calculated on the residuals after detrending the data using a smooth Gaussian kernel regression with a one-year bandwidth

(Dakos *et al.*, 2008). Time series were truncated at both ends (5%) to avoid bias at their tails (Hastie and Tibshirani 1990). EWS were then calculated within a window with length of 25% of the time series, moved over the time series one month at a time. Within the window, autocorrelation at lag-1 ρ was thus calculated using the formula for homogeneous residuals (Pinheiro and Bates 2000):

$$\bar{\rho}(l) = \frac{\sum_{j=1}^{N-l} r_j r_{j+1} / N(l)}{\sum_{j=1}^N r_j^2 / N(0)} \quad (4.2)$$

with:

l : the lag (=1 in our case)

r_j = the residuals from the generalised least square approach

$N(l)$: the number of residual pairs within the selected lag

$N(0)$: the total number of residual pair

Standard deviation was calculated with the same approach with following formula:

$$\sqrt{s^2} = \sqrt{\frac{\sum_{i=1}^N (x_i - \bar{x})^2}{N - 1}} \quad (4.3)$$

with:

s : variance

N : the total number of observations

x_i : actual residual

\bar{x} : the residual average

Lastly, we estimated the trends of both estimates up to the regime shift using the non-parametric Kendall (τ) correlation test (Kendall 1948). A positive Kendall τ means a positive trend in the estimates of EWS before the regime shift.

4.2.4 Standard Error - Robustness - Significance EWS

To test for the robustness of the trends in EWS prior to the regime shift, we divided the time series prior to the regime shift into independent blocks of 2 years length (24 monthly data points). That resulted in 19 for PCI and 14 blocks for diatom abundance. EWS were then calculated per block (block EWS) resulting in statistically independent block EWS values. We then estimated Kendall τ trends using the block EWS.

The standard deviation of the block-EWS as estimated by bootstrapping (R function: `tsboot`) the values within the blocks generating 1000 replicate block time series (Davison and Hinkley 1997). Additionally we calculated Kendall τ on the bootstrapped EWS for a given bootstrap sample (e.g. bootstrap sample 1: first bootstrapped EWS of block 1, block 2, block 3 etc.) and expressed the number of p values lower than 0.1 and 0.05 in percentage.

To determine the cut-off point at which the Kendall τ possibly gets significant, we merged the first four blocks and we added the following blocks successively one after the other. After each block addition, Kendall τ and its associated significance p value were calculated on the EWS up to the last block added. We repeated the same procedure 20 times, each time reshuffling the block order. The trends of the p values of the reshuffling were plotted together with those of the original time series.

4.3 Results

In the period from 1983 to 1989, a marked increase in PCI intensity indicated a regime shift (figure 4.2b), although such clear indication is lacking in the diatom abundance dynamics (figure 4.2c). The cumulative sum analysis, however, highlighted that a regime shift concurrently occurred around late 1980's for both PCI and diatom abundance (figure 4.2d,e).

EWS calculated within a moving window size of 25% on detrended PCI and diatom abundance, increased prior to the regime shift (figure 4.3).

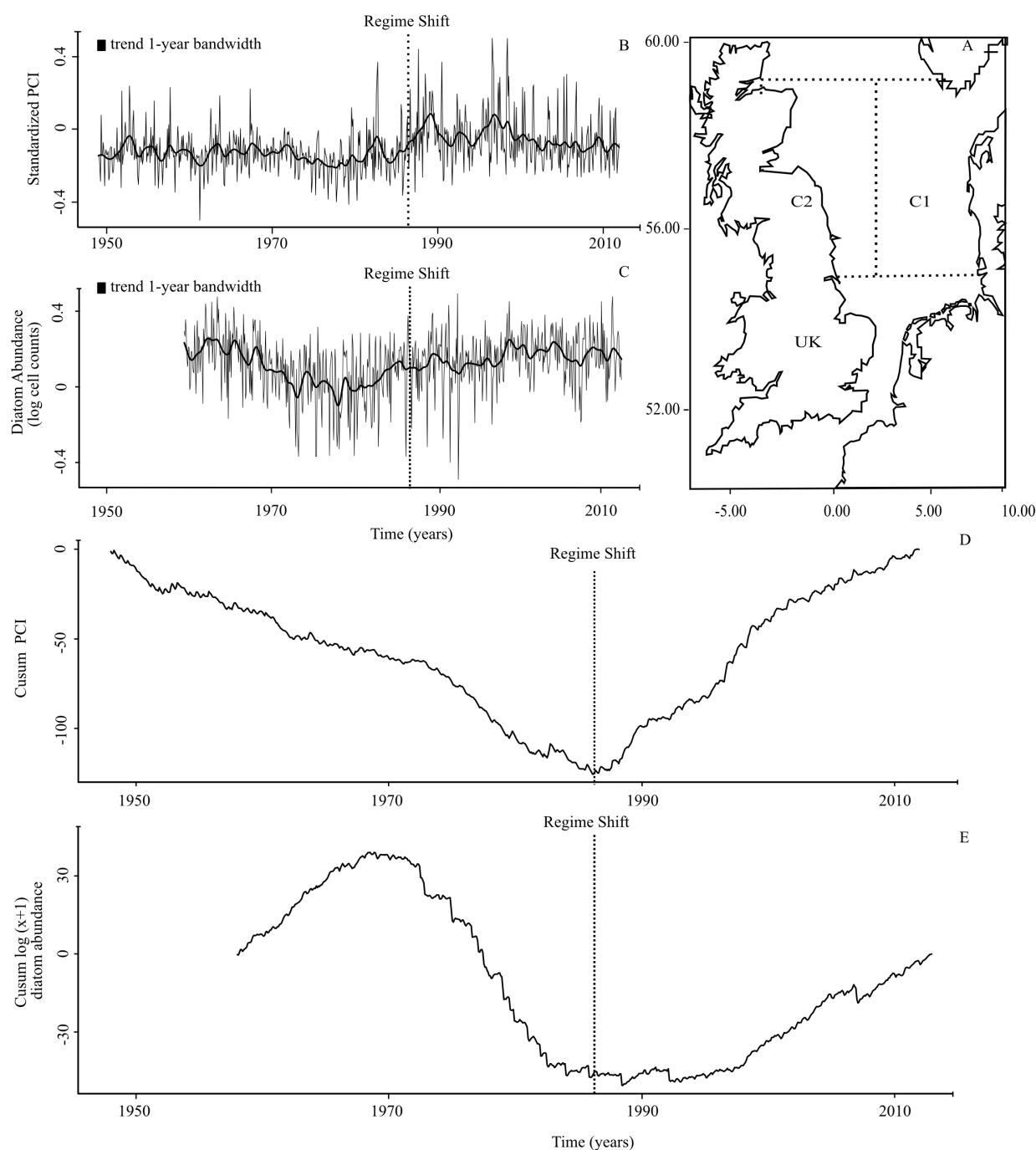


Figure 4.2: a. location of the two sample areas (C1 and C2) in the North Sea. b. monthly anomalies of standardized 'Phytoplankton Colour Index - PCI' (1948-2010) with indication of regime shift c. monthly anomalies of standardized diatom abundance (1958-2010) with indication of regime shift d. cusum (cumulative sum) of standardized 'Phytoplankton Colour Index - PCI' (1958-2010) with indication of regime shift e. cusum (cumulative sum) monthly anomalies of standardised diatom abundance (1958-2010) with indication of regime shift

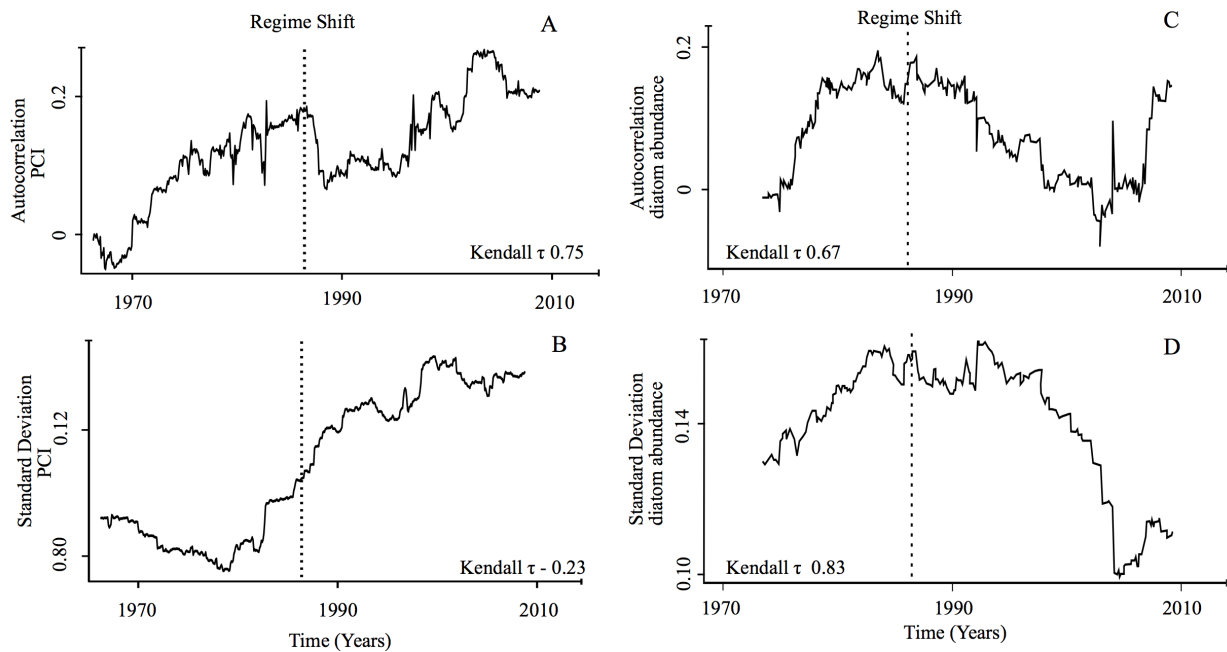


Figure 4.3: Early Warning Signals (EWS) calculated on the whole time series within a moving window size of 25% of the time series after detrending with Gaussian filtering of 1 year bandwidth. Vertical dotted line indicates the documented regime shift around 1988 a. autocorrelation at lag-1 of the standardised ‘Phytoplankton Colour Index - PCI’ (1958-2010) b. standard deviation of the standardised ‘Phytoplankton Colour Index - PCI’ (1958-2010) c. autocorrelation at lag-1 of the standardised diatom abundance (1958-2010) d. standard deviation of the standardised diatom abundance (1958-2010) In all cases, the Kendall τ was calculated from the beginning of the time series to the regime shift limit (dotted lines)

Although the increase was not monotonic, we reported significant trends for both indicators in both time series ($p < 0.001$). Autocorrelation of PCI indicated a strong increase (Kendall τ 0.80, figure 4.3a), whereas standard deviation had a negative trend (Kendall τ -0.24), although there was a clear increase prior to the regime shift (figure 4.3). For diatom abundances, we found Kendall τ trends of 0.67 and 0.82 for autocorrelation and standard deviation respectively ($p < 0.001$; figure 4.3c,d). In the case of PCI, both autocorrelation and standard deviation continued to increase after the regime shift reaching maxima in 2005 and 2000 respectively. Instead, both EWS of diatom abundance decreased after the regime shift. The trends of the EWS calculated on the independent blocks (block EWS) are shown in figure 4.4. Autocorrelation at lag-1 increased for both variables prior to the regime shift with Kendall τ of PCI of 0.47 (figure 4.4a) and 0.43 for the diatom abundance (figure 4.4c) ($p = 0.005$ and $p = 0.04$ respectively).

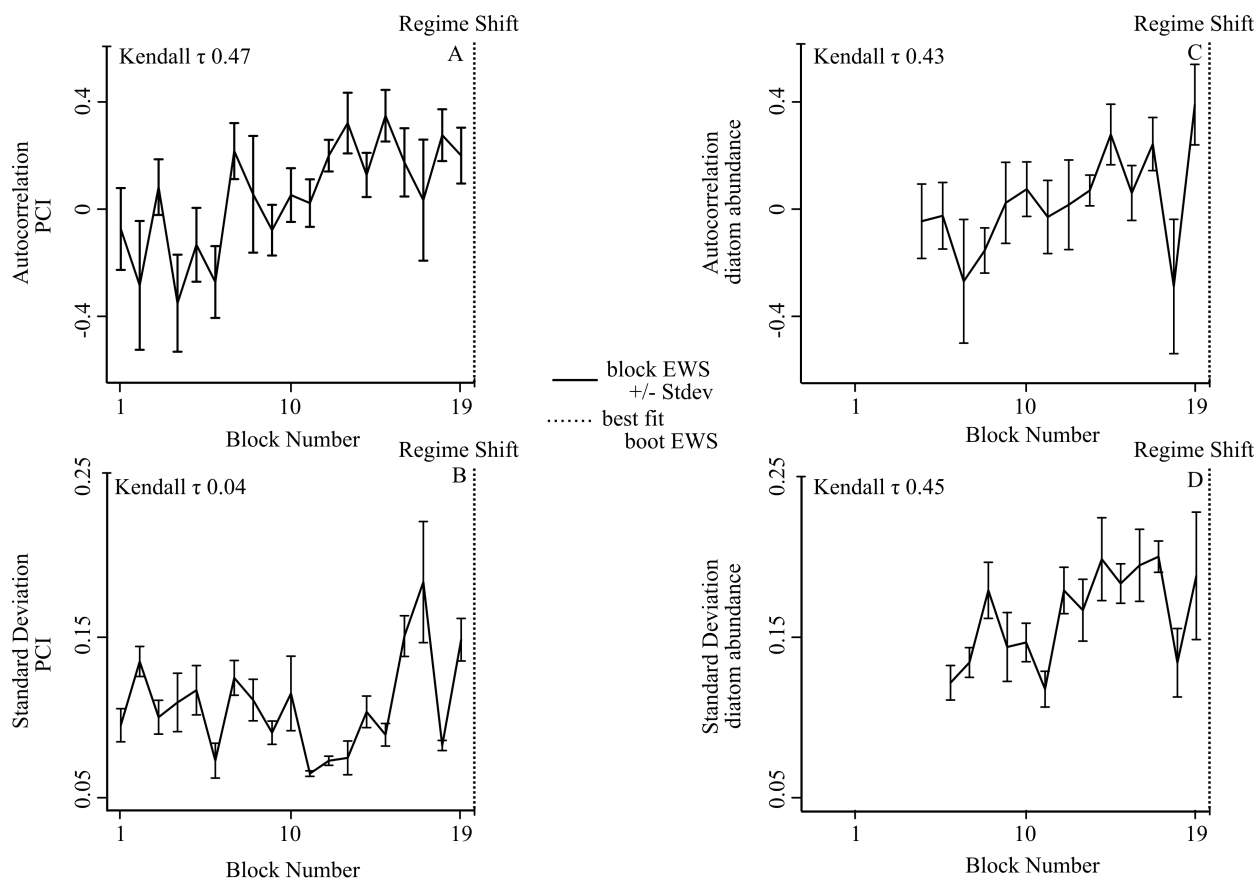


Figure 4.4: Early Warning Signals (EWS) calculated after dividing the time series prior to the regime shift into independent 2-year blocks and bootstrapping the time series within blocks. PCI data were divided in 19 blocks, whereas diatom abundances in 14 blocks. Standard deviation of 1000 bootstrapped EWS are reported as error bars (for further details see main text). a. autocorrelation at lag-1 of the standardised 'Phytoplankton Colour Index - PCI' after bootstrapping. b. standard deviation at lag-1 of the standardised 'Phytoplankton Colour Index - PCI' after bootstrapping. c. autocorrelation at lag-1 of the standardised diatom abundance after bootstrapping. d. standard deviation at lag-1 of the standardised diatom abundance after bootstrapping.

The increase in standard deviation of PCI was not significant ($p > 0.05$; figure 4.4b) while the Kendall τ for the trend of standard deviation of diatom abundance was 0.45 ($p = 0.03$; figure 4.4d). Standard deviations of the block EWS calculated on 1000 boots per block were relatively small (figure 4.4). Of the trends of the bootstrapped EWS, the autocorrelation of PCI was the most striking, with 78% and 66% of p values of the Kendall τ test being smaller than 0.1 and 0.05 respectively. For the diatom abundance 56% and 42% of p values of Kendall τ of the bootstrapped standard deviation was lower than 0.1 and 0.05 while for the autocorrelation this was 66% and 32%.

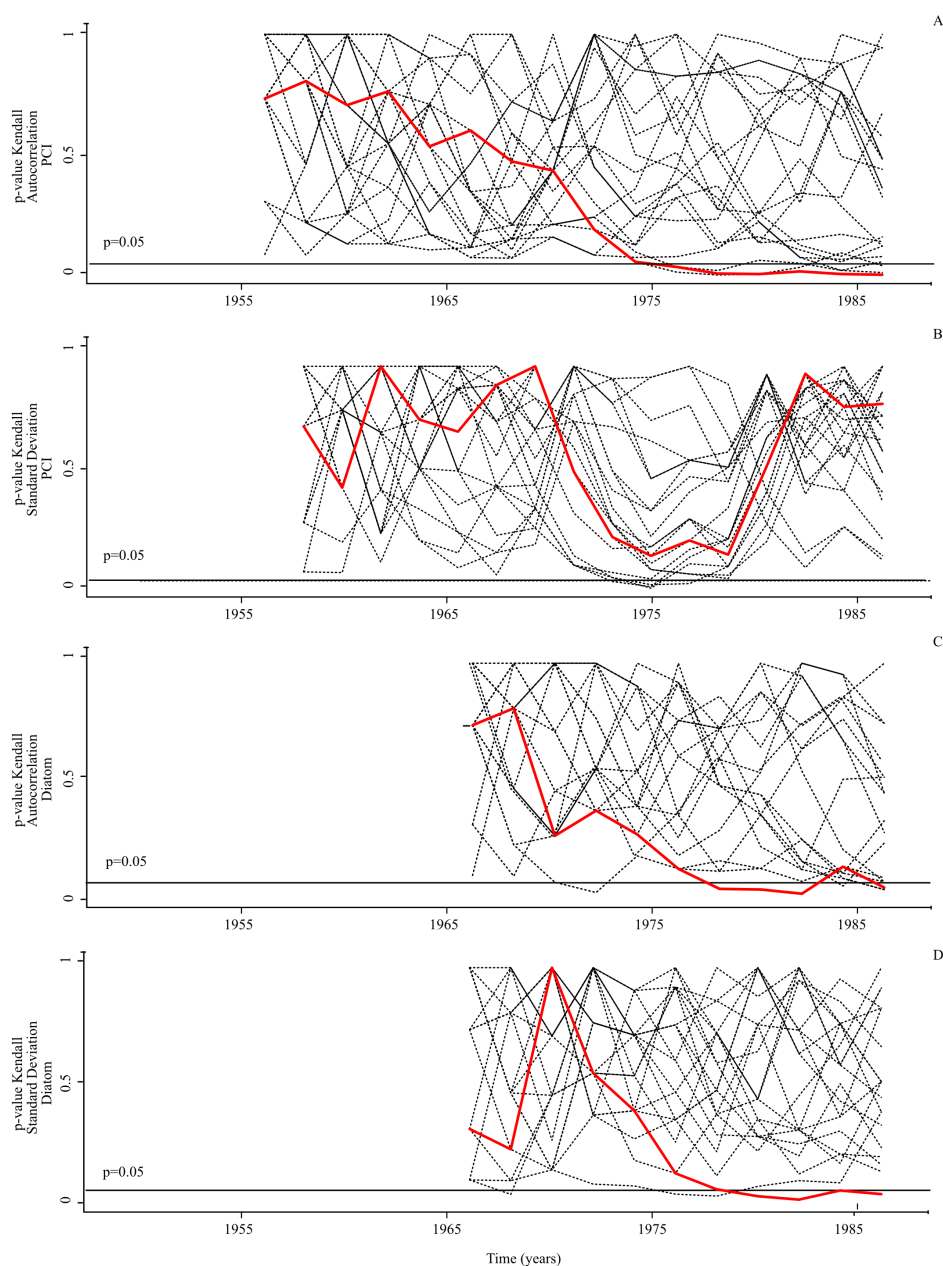


Figure 4.5: Trends of p values of Kendall τ for Early Warning Signals estimated within 2-year blocks (block EWS) (a, b) Red lines show p values on standardized PCI for autocorrelation at lag-1 and standard deviation respectively. (c, d) Red lines show p values on standardized diatom abundance for autocorrelation at lag-1 and standard deviation respectively. The procedure consists of stepwise adding blocks and iteratively calculating Kendall τ with its associated p value (for further details see main text). Dashed lines represent the p values of Kendall τ after randomly reshuffling the blocks. The horizontal line marks the p value of 0.05 significance; p values that cross this line from above mark the timing of a positive significant trend in the EWS.

The block EWS trend became significant in 1976 - 12 years prior to the regime shift - when the p value of autocorrelation at lag-1 of PCI crossed the 0.05 significance level and stayed below this level up to the regime shift (figure 4.5a). This in contrast to the trend of the p values of Kendall τ calculated on randomly shuffled blocks. In that sense, we considered year 1976 a cut-off point for the detection of the regime shift. The standard deviation of PCI approached but never crossed the 0.05 level of significance (figure 4.5b). EWS of diatom abundance also crossed the cut off in 1976 (Figure 4.5c,d), whereas no such a consistent trend of p value was found in the randomly reshuffled blocks.

4.4 Discussion

This study used two long-term phytoplankton datasets to assess whether the well-documented 1988 North Sea regime shift could have been anticipated using early warning signals for critical transitions. Using an established regime shift allowed us to test the validation of the EWS in a marine case study in retrospect. We showed that EWS picked up the increasing ecosystem instability several years in advance, and we provided a new way of assessing the robustness and significance of the warning in a case of long-term plankton time series data.

The North Sea regime shift marked a pronounced change in phytoplankton around 1988 (Reid *et al.*, 1998b). It was characterized by an exceptionally high frequency of algal blooms (Edwards *et al.*, 2006), modifications in the dominant zooplankton species (*Calanus* copepod species), and in community structure, scaling up to changes in the catches of western horse mackerel (Reid *et al.*, 2001; Reid and Edwards 2001). Various hypotheses have been proposed for the causal mechanisms of the regime shift, such as hydro-climatic forcing linked to the North Atlantic Oscillation that had probably altered nutrient budgets (Reid *et al.*, 2001). It is unclear whether the regime shift might also have been caused by a slow change in underlying conditions that progressively led the ecosystem to a state of low resilience close to a point that climatic factors might have triggered the shift (Andersen *et al.*, 2009). Our results show that increasing trends in variance and autocorrela-

tion in both time series may support the hypothesis that critical slowing down preceded the 1988 North Sea regime shift.

Nonetheless, the trends of the indicators were relatively weak when using a moving window for their calculation. Most EWS analyses based on this approach require long time series. This casts a limitation as in some cases up to half the time series needs to be used before possible trends might be picked up in EWS. As a result the opportunity of achieving an actual early warning is hindered (Lenton *et al.*, 2012b). In addition, exceptional peaks carry a significant weight on the succession of EWS values. To overcome these issues, we introduced an alternative EWS estimation based on dividing the data in statistically independent blocks. Following this approach not only we confirmed the increasing trend up to 12 years prior to the regime shift, but also we provided a way of estimating errors that can be seen as a measure of robustness for the estimation of the signals. Although this approach may prove helpful in the robust detection of EWS trends in other time series, it is still dependent on the quality of the time series (e.g. sufficient amount of data, here 24 monthly values for each block).

While we detected increasing EWS trends prior to the North Sea regime shift, our results should still be interpreted with caution (Dakos *et al.*, 2015). For instance, in the PCI abundance, the increase in EWS persists well after the onset of the regime shift contrary to the theoretically expected drop in EWS after a transition. This might relate to the fact that the regime shift persisted until the beginning of the 1990s, a period coinciding with the highest consistently positive NAO index of the 20th century (Reid *et al.*, 1998b), and that the North Sea's phytoplankton reached its theoretical threshold after 1986. It implies that the timescales under which a regime shift unfolds can be longer than expected (Hughes *et al.*, 2013), or perhaps that further forcing is being imposed on the system. A similar finding was observed in the collapse of crustacean fisheries, questioning whether the rising variability around collapse points is a transient feature as generally predicted by CSD models (Litzow *et al.*, 2013).

The few existing studies of EWS for ecosystem management in the marine environment have emphasized the potential of incorporating EWS as tools but highlighted their strong limitations (Lin-

degren *et al.*, 2012; Litzow *et al.*, 2008; Litzow *et al.*, 2013; Wouters *et al.*, 2013). Two challenges for detecting statistically significant EWS relate to the distribution of the data and also the need for replicates (Litzow *et al.*, 2013). Highly skewed data, such as population counts with values close to zero typical in the marine environment, can introduce systematic errors in EWS to be addressed with transforms. Instead, the availability of replicates adds robustness to EWS findings; for example, when catch data of multiple populations across Crustacean fisheries were pooled, EWS effectively warned 3-4 years in advance of the fisheries collapse (Litzow *et al.*, 2013). Identifying critical transitions in marine ecosystems may be more challenging than in other ecological systems. Nonetheless, in the prospect of increased uninterrupted monitoring and data gathering based on remote sensing techniques, such simple and cost effective tools should be further tested to develop a database of characteristic CSD signatures. Our work highlights how long-term, continuous marine datasets may reveal such underlying signatures and their potential use for proving the existence of critical transitions or detecting them in advance.

Acknowledgements

Noémie Wouters was funded by a PhD fellowship of Fundação para a Ciência e Tecnologia with reference ateful to Fundação para a Ciência e a Tecnologia who funded the Phd candidate (fellowship reference: SFRH/BD/48402/2008). Vasilis Dakos was supported by a Rubicon fellowship from the Netherlands Science Foundation (NWO) and a EU Marie Curie grant.

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Chapter 5

Early warning signals as indicators of
cyclostationarity in three-species
hierarchies.

Abstract

Predicting stability from current ecosystem performance is theoretically difficult, but early statistical warning signals (EWS) may enable the anticipation of regime shifts. However, little is known regarding the behaviour of EWS when the regimes are involved in cyclic dynamics. In this study, we use indicators to assess the stability of a three-species system in a competitive loop similar to a rock-paper-scissors (RPS) hierarchy. In two scenarios, the three-species interactions are simulated using a 3-D automaton whose input matrix combines pairwise dominance with differential reaction frequencies. The first scenario is based on the outcome of a microbial experiment in which the RPS hierarchy is characterized by incomplete dominance within species pairs and differences in reaction frequency between species pairs. The second artificial scenario represents a symmetric RPS interaction gradually subjected to a stressor: the reaction frequency of a species pair was modelled to decay linearly over time in favour of another species pair, while the reaction frequency of the third species pair remained constant. The species abundances are monitored spatiotemporally, and their respective equilibria are depicted on ternary plots. In the first case, the relative species abundances oscillate stably despite initial large swings, whereas in the second scenario, one species gradually dominates, eventually resulting in transitivity. In both scenarios, species cluster spatially with the development of patches of single species. In scenario 1, the average patch size remains constant throughout the iterations and possibly contributes to the overall stability; however, in the second scenario, further homogenisation takes place. Next, the effectiveness of two EWS, autocorrelation and standard deviation, as indicators of cyclostationarity is assessed. In the first scenario, both EWS were effective indicators of the system's stability in concordance with the ternary diagrams, which demonstrates species abundances settling into stable ratios. Autocorrelation detected the loss of resilience in scenario 2 with a consistent increase over iterations, whereas the standard deviation showed only an initial increase. Therefore, when cyclostationarity is lost, the amplitude change of the oscillation itself might provide a more immediate warning than standard deviation measured over an extended window.

Keywords

Automaton, patchiness, early warning signals, stability, cyclic, autocorrelation, standard deviation

5.1 Introduction

Measures that reflect stability have received considerable attention in a variety of research domains, as they have the capacity to anticipate dynamic shifts (Scheffer *et al.*, 2009). To this end, one approach uses statistical measures, termed early warning signals (EWS), such as autocorrelation and variance (Scheffer 2010). These indicators represent a promising approach to resilience assessment, as they render its concept operational (Rougé *et al.*, 2013). EWS also meet the criteria considered crucial for sound ecological indicators, such as ease of calculation and anticipation of reduced system integrity (Dale and Beyeler 2001). Increases in EWS indicate a slowing down of the system, a phenomenon occurring frequently prior to dynamic shifts (Lenton *et al.*, 2012). When subsequent states in a time series become more alike, the autocorrelation at low lags (short-term memory) increases and the recovery from small perturbations slows down (Scheffer *et al.*, 2009; Scheffer 2010). Additionally, prior to a transition, the variance of the fluctuations (hence the standard deviation) also increases (Dai *et al.*, 2012). In biology, with the exception of cyclic shifts between alternative states, the reading and behaviour of EWS on cyclic systems has received limited attention despite their common occurrence in nature (van Nes *et al.*, 2007). Such dynamics can result from external cyclic forcing such as glaciations (Petit *et al.*, 1999), or from internal dynamics where, indeed, EWS can predict bifurcations in two-species predator-prey models (Chisolm and Filotas 2009). EWS have rarely been exploited in models with cyclic dynamics (Beninca *et al.*, 2009), and to the best of our knowledge, they have not yet been applied to cases in which more than two species interact in competitive loops, as frequently observed in ecosystems (Petraitis 1979). However, in other domains, particularly communication and signal processing, EWS-type statistical functions have been used extensively to monitor cyclostationarity (Gardner *et al.*, 2006). Cyclic phenomena represent regular fluctuations around a basin of attraction but might become subject to a transition to another attractor. Scheffer *et al.*, (2009) mention that the dynamics of harmonic systems, aside from having different types of bifurcations, may be expected to slow down before a critical transition and are thus likely to be characterized by EWS when a stressor forces the system to the boundary of its basin of attraction. In addition to autocorrelation and variance, particular spatial heterogeneity can also signal an upcoming regime shift as has been observed in vegetation

patterns: before a critical transition into a barren state, the pattern of vegetation changes from a maze-like type to a more connected structure with isolated spots (Scheffer *et al.*, 2009; Rietkerk *et al.*, 2004). Patchiness, a fundamental property of most benthic communities, is dynamically influenced not only by biological factors such as predation and foraging patterns but also by stochastic and physical disturbances (Burrows and Hawkins 1998). It is a crucial element in other realms; for example, bacteria typically show cohesive habitat associations with units of antibiotic producing, resistant or sensitive strains (Cordero *et al.*, 2012; Kirkup and Riley 2004; Morlon 2012). Although regular spatial patterns persist in environmental heterogeneity, there is a general tendency towards increased spatial coherence -measured as increased cross-correlation among units - with an increase in the size of single-species patches before shifts (Burrows and Hawkins 1998; Scheffer *et al.*, 2009).

Biological systems are often expressed in the form of a network (Fath 2004). With only two species, the rule defining their hierarchy is represented by the coin-toss model (Kroese *et al.*, 2011). When three species are present, this opens up the possibility of a competitive cycle in which species pairs follow a defined hierarchy but the entire system is non-transitive (Petraitis 1979). One of the simplest examples of cyclic dominance is the children's game rock-paper-scissors (RPS), in which a rock beats a pair of scissors, scissors beat a sheet of paper and paper beats a rock (Frean and Abraham 2001). RPS systems are of great interest in various disciplines, as they are dynamically stable and characterized by oscillations, and as such allow great diversity that is improbable under transitive hierarchies (Frean and Abraham 2001; Allesina and Levine 2011). In marine biology, coverage and overgrowth of sessile organisms on rocky shores and Jamaican coral reef showed this type of cyclic competitive structure (Buss and Jackson 1979; Burrows and Hawkins 1998). In bacterial research, RPS interactions studied by combining mobility of species and the frequency of their reactions were found to favour the sustainability of bacterial diversity (Venkat and Pleimling 2010; Cordero *et al.*, 2012; Morlon 2012). *In vivo* experiments carried out on the bacterial population of the intestines of caged mice also resulted in a RPS interaction (Kirkup and Riley 2004). The stability of RPS is determined by the pair-wise dominance between species, which can be estimated by the observed proportion of wins of a species against the other. In addition, differences in reaction frequencies between species pairs might also be influential. Indeed, certain

models account for this factor by considering reaction frequencies as probabilities proportional to the overall species densities (Frea and Abraham 2001). Different interaction rates can result in the transition of coexisting species to a regime where only one species eventually survives (Venkat and Pleimling 2010). In field observations and predator-prey models of rodents, there is clear evidence that changes in temperature and humidity altered encounter opportunities and resulted in a loss of stability (Kausrud *et al.*, 2008).

The empirical estimation of the strength of competition and the assessment of the degree of competitive intransitivity in nature have proven difficult (Ulrich *et al.*, 2014). The use of automata to simulate the stability of networks is well established (Baianu 1985). Simulation of a spatial automaton describing non-transitive hierarchies for species associations of 3 up to 25, found that (a)symmetry of the hierarchy due to differences in competitiveness often relates to the stability of the systems (Laird and Schamp 2006). The input for a cellular automaton derived from 45-year monitoring of the interaction of fucoids, barnacles and limpets allowed the study and simulation of their spatiotemporal evolution (Burrows and Hawkins 1998). Experimental data on bacterial diversity in petri dish and mice intestines are also consistent with simulations by a 2-D automaton (Kerr *et al.*, 2002; Durrett and Levin, 1998). In short, automata allow simulation of system dynamics conditioned on the starting populations. Using automaton simulations, we address the effectiveness of EWS to detect the loss cyclosationarity in the dynamics of RPS hierarchy in two scenarios.

5.2 Materials and Methods

5.2.1 The automaton model

Two three-species hierarchies are considered in an automaton consisting of a $27 \times 27 \times 27$ cube. Inside this array, $25 \times 25 \times 25$ compartments each contains one of three species. Starting with a checkerboard pattern, the species react through iterations along the coordinates of the array with their six neighbour species (figure 5.1).

The reactions are computed according to probability rules set forth in the matrices defined below. Take three species 1, 2 and 3 competing pair-wise $\{u\}$ versus $\{v\}$ where $\{u\}$ and $\{v\}$ can be species 1, 2 or 3. The probability of any species replacing another species is determined by two factors:

(a) The rule $r_{u,v}$ is the probability of $\{u\} > \{v\}$, given the occurrence of a reaction between $\{u, v\}$.

Hence $r_{u,v} = 1 - r_{v,u}$.

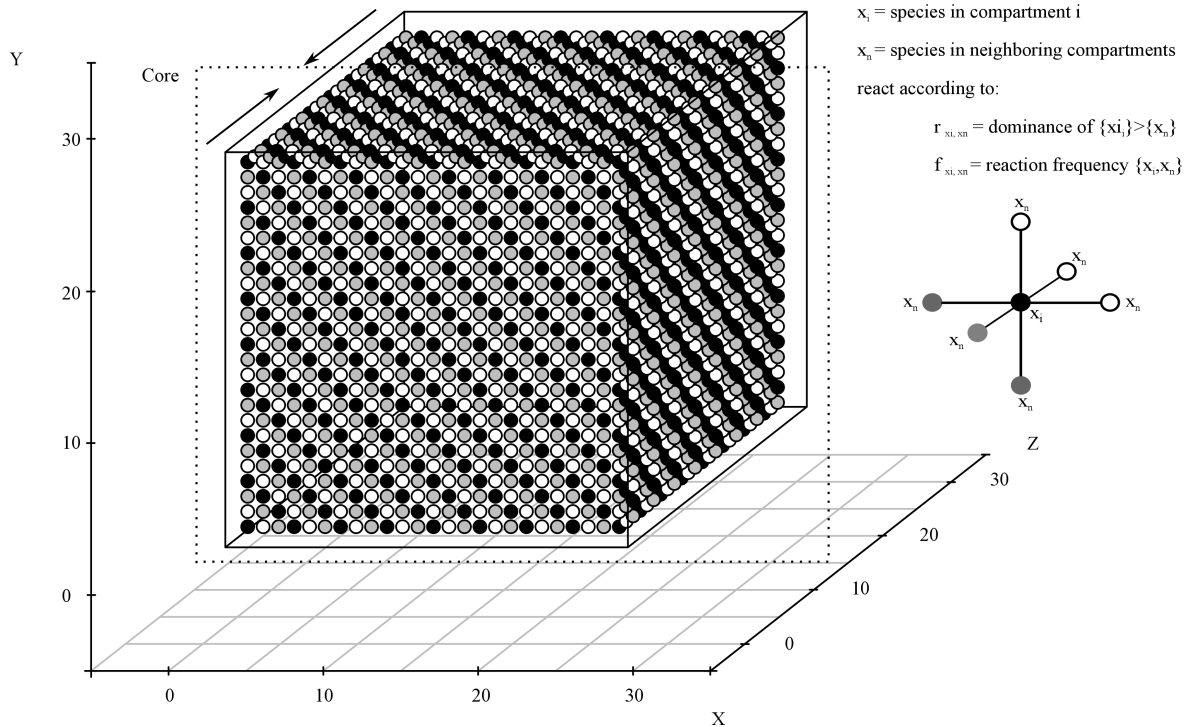


Figure 5.1: The 3-D cube of the automaton initiating with a checkerboard pattern, at each iteration species x_i interact with their 6 neighbours x_n according to predefined ruling and reaction frequency (for details see material and methods). Indication of a cross section in the middle of the cube (core) used for calculation of the patchiness index.

(b) The reaction frequency $f_{u,v}$ weighs the rules by defining how often a given pair $\{u, v\}$ reacts, relative to the average reaction rate.

Hence $f_{u,v} = f_{v,u}$, and the average frequency of the reactions line $\{u\}$, column $\{v\}$, of the matrix is thus set to one. For three species, this can be represented mathematically by two matrices: $R = |r_{u,v}|$ and $F = |f_{u,v}|$:

$$R = \begin{bmatrix} 1 & r_{1,2} & r_{1,3} = 1 - r_{3,1} \\ r_{2,1} = 1 - r_{1,2} & 1 & r_{2,3} \\ r_{3,1} & r_{3,2} = 1 - r_{2,3} & 1 \end{bmatrix} \quad (5.1)$$

$$F = \begin{bmatrix} 1 & f_{1,2} & f_{1,3} = f_{3,1} \\ f_{2,1} = f_{1,2} & 1 & f_{2,3} \\ f_{3,1} = 3 - f_{1,2} - f_{2,3} & f_{3,2} = f_{2,3} & 1 \end{bmatrix} \quad (5.2)$$

If adjacent species are identical, the same species will inherently occupy the compartment after a reaction, hence a probability of 1 in the diagonal of the R matrix. This is independent of the reaction frequency; hence by convention the diagonal of F is also set to 1. Multiplying matrix F element-wise by matrix R sets the probability of the possible interactions: six reactions within different species pairs and three same species interactions (diagonals). The elements of the matrix are divided by 6 to sum up to a probability of 1:

$$P = \begin{bmatrix} 1/6 & f_{1,2}r_{1,2}/6 & f_{1,3}r_{1,3}/6 \\ f_{2,1}r_{2,1}/6 & 1/6 & f_{2,3}r_{2,3}/6 \\ f_{3,1}r_{3,1}/6 & f_{3,2}r_{3,2}/6 & 1/6 \end{bmatrix} \quad (5.3)$$

In the automaton, the probability that a given species will remain or be replaced is a function of the surrounding species and the fitness matrix P .

The probabilities of species x_i at iteration T in compartment i being one of the three species a_1 , a_2 or a_3 sums up to 1:

$$Pr(x_i^T = a_1) + Pr(x_i^T = a_2) + Pr(x_i^T = a_3) = 1 \quad (5.4)$$

The calculation of the probabilities for each species is modelled as a two step process. Let n represent the six compartments surrounding i . The probability of species x_i^T being a_i^T depends on whether a_i was present in that compartment at iteration $T-1$ or not. If $x_i^T = a_i^T$, the probability of species x_i^T remaining in place is governed by overcoming the possible reactions with its neighbouring species x_n^T in any of the six compartments surrounding i . The within-pair probabilities determined by rule r_{a_i, x_n} , and weighted by the relative frequency of reactions f_{a_i, x_n} are added up around the six reactions between neighbours and divided by the total frequency of possible reactions $\sum_n f_{a_i, x_n}$.

In short, given that a_i was present in compartment i at $T-1$, the probability that the same species occupies this compartment i at T is computed as in 5.5

$$Pr(x_i^T = a_i | x_i^{T-1} = a_i) = \frac{\sum_n f_{a_i, x_n} \cdot r_{a_i, x_n}}{\sum_n f_{a_i, x_n}} \quad (5.5)$$

In contrast, if compartment i was not occupied by a_i at iteration $T-1$, $Pr(x_i^T = a_i^T)$ represents the probability of a species of one of the surrounding compartments a_n invading compartment i . In this case the probabilities are determined by rule of r_{a_i, x_i} weighted by the frequency f_{a_i, x_i} . In short, this probability is computed via 5.6, where summation is over the six neighbouring compartments divided by the total frequency of possible reactions.

$$Pr(x_i^T = a_i | x_i^{T-1} \neq a_i) = \frac{\sum_n f_{a_n, x_i} \cdot r_{a_n, x_i}}{\sum_n f_{a_i, x_n}} \quad (5.6)$$

The iterations are thus conditioned by the outcome of the previous round of reactions. For both scenarios specified below, 500 iterations were run and three replicas were generated. The automaton was developed in .R software, version 3.0.1 (.R Development Core Team 2014). After each iteration, the automaton reported on relative species abundance visualised by cumulative plots. To obtain an overall picture of the scenario's dynamics with a visualisation of the basins of attraction of the hierarchies, the ratios of the species abundances were graphically depicted as positions in equilateral triangles on ternary plots as described by (Venables and Ripley 2002; Frean and Abraham 2001).

To assess the spatial distribution of the species, an index of patchiness was calculated as described

by Kefi *et al.*, 2007. Species aggregation was analysed on the core of the cube: a cross-section of the center (figure 5.1). For each transect of this core, the number of compartments belonging to the same species without discontinuity was counted relative to the total number of compartments of the cross-section. The initial checkerboard therefore has a patchiness index of 0%, and if only one species remains, the patchiness is 100%.

5.2.2 Two scenarios as input for automaton

The respective input matrices for the two automaton scenarios are defined below. The first scenario used the competitive outcome based on numbers of wins of an *in vivo* bacterial experiment with three strains of *E. coli*. A detailed description of the set-up of the experiment is found in Kirkup and Riley 2004. The interaction of the bacteria, of which RPS interaction was confirmed by *in vitro* experiments and a 2-D model (Kerr *et al.*, 2002), was studied in the intestine of three caged mice interacting freely. The experiment ran for 12 weeks with two observations per week in a total of 12 cages. The probability matrices of the unequal returns and the different reaction frequencies between species pairs correspond to:

$$\text{Rule } R = \begin{bmatrix} 1/6 & 0.714/6 & 0.362/6 \\ 0.286/6 & 1/6 & 0.783/6 \\ 0.638/6 & 0.217/6 & 1/6 \end{bmatrix} \quad (5.7)$$

$$\text{Reaction frequency } F = \begin{bmatrix} 1 & 1.333 & 1.119 \\ 1.333 & 1 & 0.548 \\ 1.119 & 0.548 & 1 \end{bmatrix} \quad (5.8)$$

$$\text{Resulting in fitness matrix } P = \begin{bmatrix} 0.167 & 0.159 & 0.067 \\ 0.063 & 0.167 & 0.071 \\ 0.119 & 0.020 & 0.167 \end{bmatrix} \quad (5.9)$$

For the second, artificial scenario a symmetric RPS where the dominance within each species pair always follows a given direction, was considered 5.10. Equal reaction frequencies between species pairs were used 5.11 and the input matrix for the automaton was 5.12. However the $f_{1,2}$ of F

was modelled to progressively decay in favour of $f_{1,3}$ while $f_{2,3}$ remained constant. The decay diminished with a factor of 1/500 every iteration, to result in zero at iteration 500.

$$\text{Rule } R = \begin{bmatrix} 1/6 & 1/6 & 0 \\ 0 & 1/6 & 1/6 \\ 1/6 & 0 & 1/6 \end{bmatrix} \quad (5.10)$$

$$\text{Reaction frequency } F = \begin{bmatrix} 1 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 1 \end{bmatrix} \quad (5.11)$$

Resulting in the following fitness matrix for automaton at iteration 1:

$$P = \begin{bmatrix} 0.167 & 0.167 & 0 \\ 0 & 0.167 & 0.167 \\ 0.167 & 0 & 0.167 \end{bmatrix} \quad (5.12)$$

5.2.3 Spatiotemporal early warning signals

The autocorrelation and standard deviation were calculated on the log-transformed abundance of species 1. The methodology was based on Lenton *et al.*, (2012). After de-trending from the slope by fitting a linear model using generalised least squares, the lag-1 autocorrelation was calculated on the residuals, defined as the difference between the de-trended and smoothed signal calculated with a Gaussian kernel regression smoother (bandwidth = 6% of the time series length). The time series were also truncated at both ends (5%) to avoid bias at their tails (Hastie and Tibshiran 1990). The autocorrelation and standard deviation were calculated on a window of the time series (35%) automatically moved over the time series sample, one step at a time. The lag-1 autocorrelation was used (Pinheiro and Bates 2000).

$$\bar{\rho}(l) = \frac{\sum_{j=1}^{n-l} r_j r_{j+1} / N(l)}{\sum_{j=1}^n r_j^2 / N(0)} \quad (5.13)$$

with:

l: the lag

r_j = the residuals

$N(l)$: the number of residual pairs within the selected lag

$N(0)$: the total number of residual pair

Standard deviation was calculated as follows:

$$SD = \sqrt{\frac{\sum_{i=1}^N (x_i - \bar{x})^2}{N - 1}} \quad (5.14)$$

with:

N : the total number of observations

x_i : the log abundance of species 1

\bar{x} : the average of log abundance of species 1 over the time series

To evaluate the EWS trends the tau (τ) statistic for the non-parametric Kendall test was used (Kendall 1948; Lenton *et al.*, 2012).

5.3 Results

5.3.1 Stability of the two scenarios

Starting with identical relative species abundances as imposed by the checkerboard pattern, the abundances in the two scenarios vary cyclically (figure 5.2). Initially, scenario 1 is characterized by oscillations of great amplitude. After 25 oscillations species 3 is the most abundant (85%): the high reaction frequency between species 1 and 2 and the dominance of species 1 over species 2 result in an initial decrease of abundance of species 2, allowing species 3 to increase. After 75 iterations the abundance of species 2 increases to 40% and species 3 drops to 60% while remaining the most abundant species. The initial instability dampens out while the harmonic variations persist with a maximum variation of 20% in the abundance of the three species. The ternary plot confirms this evolution: starting off in the center (checkerboard), the ratio circles towards a dominance of species 3, with ratios of 0.85 and 0.08 for species 3:1 and 3:2.

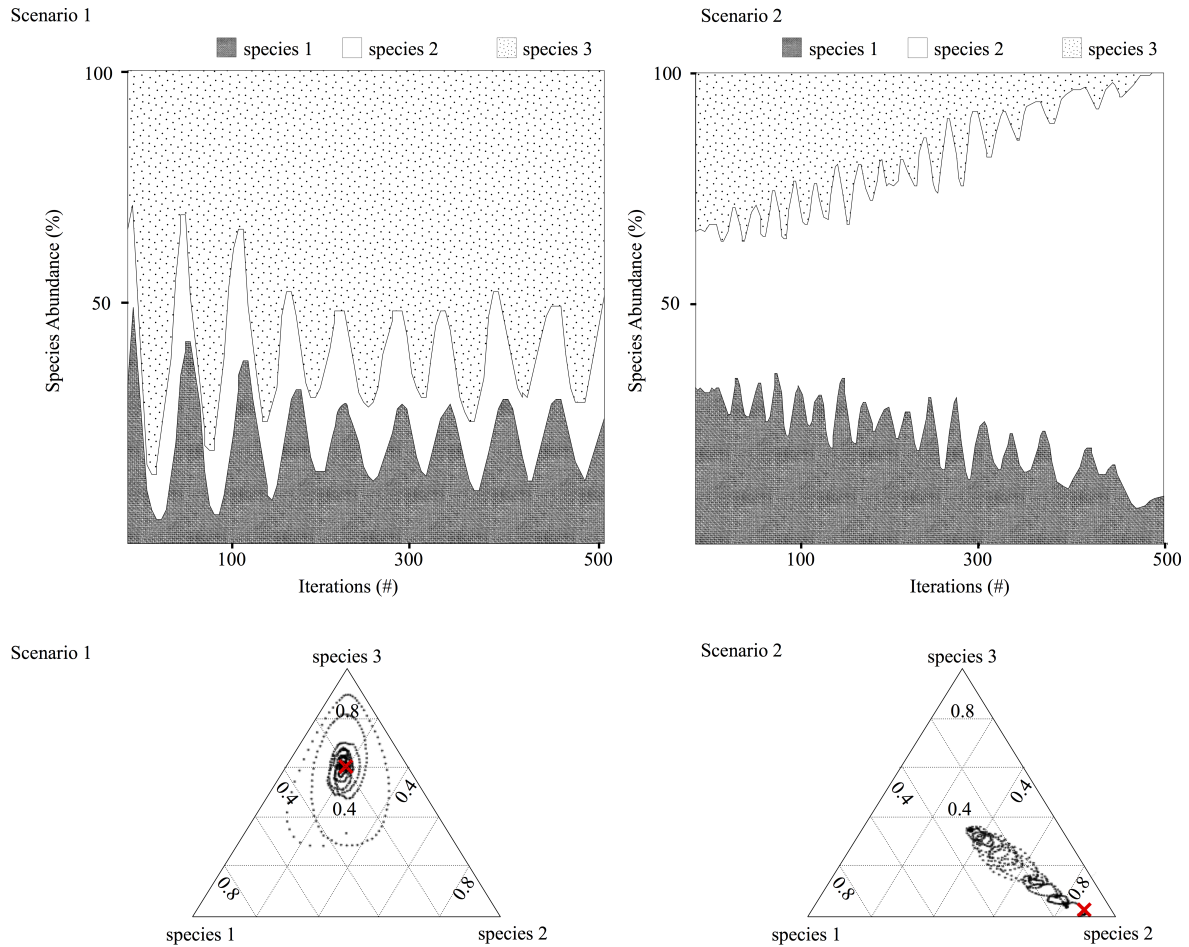


Figure 5.2: The relative abundance (%) of the three species throughout the iterations in scenario 1 (asymmetric RPS hierarchy) and in scenario 2 (RPS hierarchy subjected to gradual decrease in the frequency of encounter between species 1 and 2). Ternary diagrams of the respective scenarios with the end of the iterations shown as a red cross.

However, afterwards, the oscillations eventually settle into an internal equilibrium point with a stable ratio of 0.2:0.2:0.6 for species 1:2:3 (figure 5.2). In scenario 2, the harmonic variations in the species abundances never exceed 10% but species 2 progressively dominates the system and, in the end, out-competes the two other species. The decrease in frequency of encounter between species 1 and species 2 combined with the increase in reaction frequencies between species 1 and species 3 results in their disappearance in favour of species 2. Ternary plots confirm this evolution: starting from the fixed point reflecting the checkerboard distribution, the ratio gradually spirals to complete dominance by species 2. The replicates of both scenarios are consistent with the above (sup. 1).

As for the habitat heterogeneity, in both cases the patchiness index starts at 0%: no patches (figure 5.3). In scenario 1 the percentage of continuous cover increases to 60% and, despite some oscillations, remains this high for the remaining iterations. In scenario 2, despite some oscillations, the patchiness index linearly increases to 100%. The same is observed for the replicates of both scenarios (sup. 2).

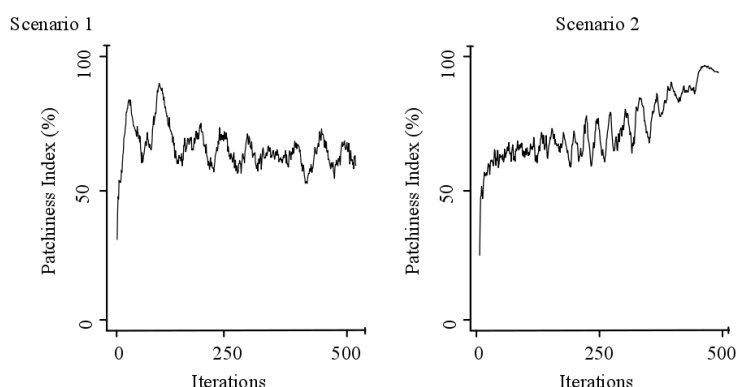


Figure 5.3: Patchiness index, continuous same-species coverage (%) over the core throughout the iterations in scenario 1 (asymmetric RPS hierarchy) and in scenario 2 (RPS hierarchy subjected to gradual decrease in the frequency of encounter species pairs 1 and 2).

5.3.2 Early warning signals

In both cases, the trend in autocorrelation shows cyclic variations as the species abundance (figure 5.4). In scenario 1 the lag-1 autocorrelation remains stable and is characterized by a low Kendall τ (-0.29) thus reflecting the overall stable abundance of species 1. The standard deviation also reflects this cyclostationarity, with a slight decrease and a low Kendall τ (-0.24).

Similar observations were made for the replicates of scenario 1, with both early warning signals indicating stability (sup. 3).

In scenario 2 the lag-1 autocorrelation shows a consistent increase from 0.26 to 0.47 with Kendall $\tau = 0.90$, thus reflecting the increasing instability of the hierarchy. The standard deviation on the other hand initially increases from 0.15 to 0.22, indicating instability, but afterwards declines again with Kendall $\tau = -0.27$. Both replicates of scenario 2 show the same patterns for both early warning signals (Sup. 3).

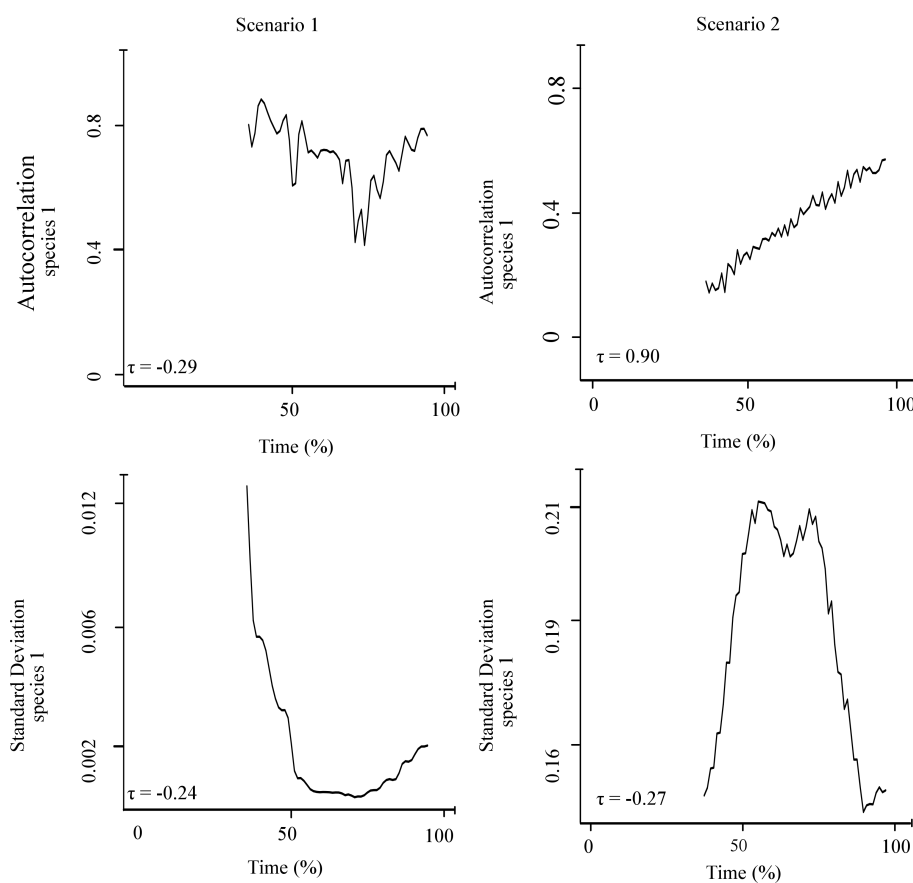


Figure 5.4: Early warning signals (autocorrelation and standard error) calculated on the detrended relative log-abundance of species 1 with bandwidth 6,5% and window size 35% in scenario 1 (asymmetric RPS hierarchy) and in scenario 2 (RPS hierarchy subjected to gradual decrease in the frequency of encounter species pairs 1 and 2).

5.4 Discussion

5.4.1 Cyclostationarity of RPS hierarchies

In a scenario in which species 1 outcompetes species 2 and 3, and species 2 outcompetes species 3, the three species can be listed unambiguously in order of their competitive abilities ($1 > 2 > 3$) and the outcome is a transitive hierarchy. However, in a RPS system, 1 outcompetes 2, 2 outcompetes 3, and 3 outcompetes 1 ($1 > 2 > 3 > 1$) and the resulting hierarchy is intransitive. Conceptually, the dynamic fluctuations associated with this cyclic competitiveness can be understood as follows: a lower abundance of 1 means a lack of 2's only dominator and thus 2 has a competitive advantage,

increases its abundance and gradually replaces 3. When the abundance of 3 decreases, 1 gets an advantage and starts to replace 2. Once 2 almost disappears 3 lacks a competitor and increases in abundance, which in turn yields the decrease of 1 and the whole cycle repeats again (MacMartin and Rychtar 2007). Given the odd number of species in the competitive loop, a lowered invasion rate for one species leads to a decrease in the population of the species that invades it (Frean and Abraham 2001). Despite being seemingly trivial, these cyclic interactions emerge frequently in nature and ensure stability (Jiang *et al.*, 2011). For example, such interactions are common in marine sessile organisms, as well as in the bacterial realm (Frean and Abraham 2001; Burrows and Hawkins 1998). More specifically, *in vitro* and *in silico* experiments using three strains of *E. coli* - a toxin-producing, a toxin-resistant and a toxin-sensitive strain - satisfied a RPS relationship (Kerr *et al.*, 2002). In an experiment in which the bacterial population dynamics in the intestines of mice infected with one of the three strains were analysed, a discrete RPS pattern was observed (Kirkup and Riley 2004). Here, we use the competitive outcome of this experiment as inputs for a 3-D automaton and assess the stability of the hierarchy characterized by unequal returns (no complete dominance) and differences in reaction frequency. Previously, a 2-D model of this system determined the relative fitness of the strains based on the outcome of pairwise competition *in vitro* and assumed complete dominance (Kerr *et al.*, 2002). Other authors also did not account for relative differences in competitive reversals while assessing the stability of RPS (Frean and Abraham 2001). An approach in which reverse engineering is used to estimate the degree of intransitivity of multi-species interactions only identifies within-pair competitive strength and assumes equilibrium at all times while acknowledging it is essential to address these aspects of the dynamics (Ulrich *et al.*, 2014). The design of the input probability matrices in our model allows to adequately account for observed asymmetries. Within a pair, the odds of dominance might be different from 0/1, as was the case in our first scenario. Furthermore, our model enables to take into account differences in reaction frequency between pairs observed experimentally and it can take into account dynamic shifts in the degree of transitivity (scenario 2).

Finally our automaton is modelled in 3-D and thus accurately reflects the biotic environment of the bacteria. Based on the empirically derived fitness, the asymmetric RPS could be stable in the long run whereby, after initial large oscillations, the species abundances show a regular cyclicity. The

initial instability in our model before the RPS settles in its basin of attraction can be attributed to both the short course of the experiment and its small population size, which predisposed the *in vivo* experiment to instability (Kirkup and Riley 2004). The second scenario, an initially symmetrical RPS (complete dominance) with the reaction frequencies of a given pair gradually modelled to decrease in favor of another pair, was unstable over time. Species abundances in both scenarios are characterized by sinusoidal patterns, typical for RPS interactions as the feed-forward loop regulates changes in abundance (MacMartin and Rychtar 2007). These regular oscillations have been observed both empirically and in models of mating strategies of side-blotched lizards (Sinervo and Lively 1996). On ternary plots the population densities of a symmetric RPS hierarchy form closed orbits around a fixed point (Frean and Abraham 2001). This graphical representation allows for the visualisation of the basin of attraction of RPS hierarchies in the same way as the plots used in search of alternative stable states in coral reef dynamics, where the benthic time series was categorised in three components (Zychaluk *et al.*, 2011). Our first scenario eventually encounters its equilibrium, characterized by a greater abundance of one of its species, while in the second scenario the system spirals away from its equilibrium point. We demonstrate that different reaction frequencies indeed influence dynamic stability of cyclic competition, and might even end-up in transitivity. This is consistent with previous results that highlight the law of the weakest predator surviving, is replaced by different dynamic regimes (Venkat and Pleimling 2010). Similarly, influencing the intensity of species reactions in a 2-D RPS model by adding the diffusion of a toxin eventually results in a monoculture (MacMartin and Rychtar 2007).

The cyclostationarity of RPS has been linked to specific spatial structures: in RPS models with unequal invasion rates a variety of spatial structures was observed (Frean and Abraham 2001). Here, the spatial structure of the asymmetric RPS is characterized by species clusters as previously evidenced *in vivo* and *in silico* (Kirkup and Riley 2004, MacMartin and Rychtar 2007). This social patchiness has been hypothesized as a survival strategy in adverse conditions (Kerr *et al.*, 2002). In scenario 2, in which the RPS becomes ultimately transitive, we observed a further increase in patch size, leading logically to a homogenisation reflecting dominance by one species. A transient patchiness also slowly decayed in a two-species model in which either species is able to prevent the other from colonising (Caswell and Etter 1999).

5.4.2 Early warning signals and cyclostationarity

Cyclic phenomena are frequently observed in ecology at different orders of magnitudes: in climatological phenomena such as the North Atlantic Oscillation, in predator-prey interactions such as the typical phytoplankton–zooplankton oscillations (Beninca *et al.*, 2009) and in RPS interactions showing distinct sinusoidal patterns (Frean and Abraham 2001). Whereas EWS have previously been assessed in two-species competition models with bifurcations and a transition (Chisholm and Filotas 2009), to our knowledge they have not yet been used to assess cyclostationarity of RPS hierarchies with three agents. Here, we use two scenarios of RPS hierarchies as models to explore the behaviour of EWS in such dynamics. Typically, increasing trends in EWS are used as signals of instability (Dakos *et al.*, 2012). In scenario 1, the asymmetric RPS interaction, the initial decrease in EWS might be linked to the disappearance of the major oscillations in species abundance as the system reorganises itself from the homogeneous checkerboard to a patched structure, with abundance compensating for the differences in fitness. Eventually, both EWS remain constant and reflect the cyclostationarity associated with the system settling into its basin of attraction (Gardner *et al.*, 2006).

Linear systems with smooth potentials are more likely to be characterized by leading EWS indicators, as demonstrated in the case of a three-species food chain in which all species migrate between six patches (Hastings and Wysham 2010). However, Scheffer *et al.*, (2009) mention that, when harmonics lose resilience, they might also reflect leading indicators. In scenario 2, the progressive reduction of reaction frequency for one of the species pairs in favor of another pair introduces a smooth potential to the cyclic system and gradually forces the RPS to instability. In this case, the decrease in resilience is picked up by early warning signals. However, in one case, the standard deviation did not accurately reflect the loss of cyclostationarity. Here, a direct measurement of the amplitude of sine waves might provide a more immediate indication of the resilience loss, as the window used in the calculation of EWS introduces a lag in their response to instability. Overall, our results indicate that EWS are useful tools to detect a loss of cyclostationarity. Moreover, their calculation is relatively easy compared to more complex approaches such as wavelet analysis, which was previously used to assess the stability of cyclic predator–prey interactions (Beninca *et al.*, 2009). Whereas patchiness has been used as an EWS to predict two-species regime shifts (Kefi

et al., 2007), it is a challenge to identify it as EWS in RPS hierarchies. In fact, the patchiness observed in our two scenarios might actually be a contributor to the stability of the system as a whole (Kerr *et al.*, 2002). As a final note, we highlight the relevance of our conceptual three-species model for ecosystem networks: while food-web networks are complex, their actual flow tends to concentrate within three dominant nodes (Borrett 2013). Therefore, EWS applied to the fluctuations of populations at two or three major nodes might serve as indicators of stability loss even if cyclic trends are present, as illustrated in this paper.

Acknowledgements

The first author was funded by a PhD fellowship of Fundação para a Ciência e a Tecnologia with reference ateful to Fundação para a Ciência e a Tecnologia who funded the Phd candidate (fellowship reference: SFRH/BD/48402/2008).

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5.5 Supplementary Material

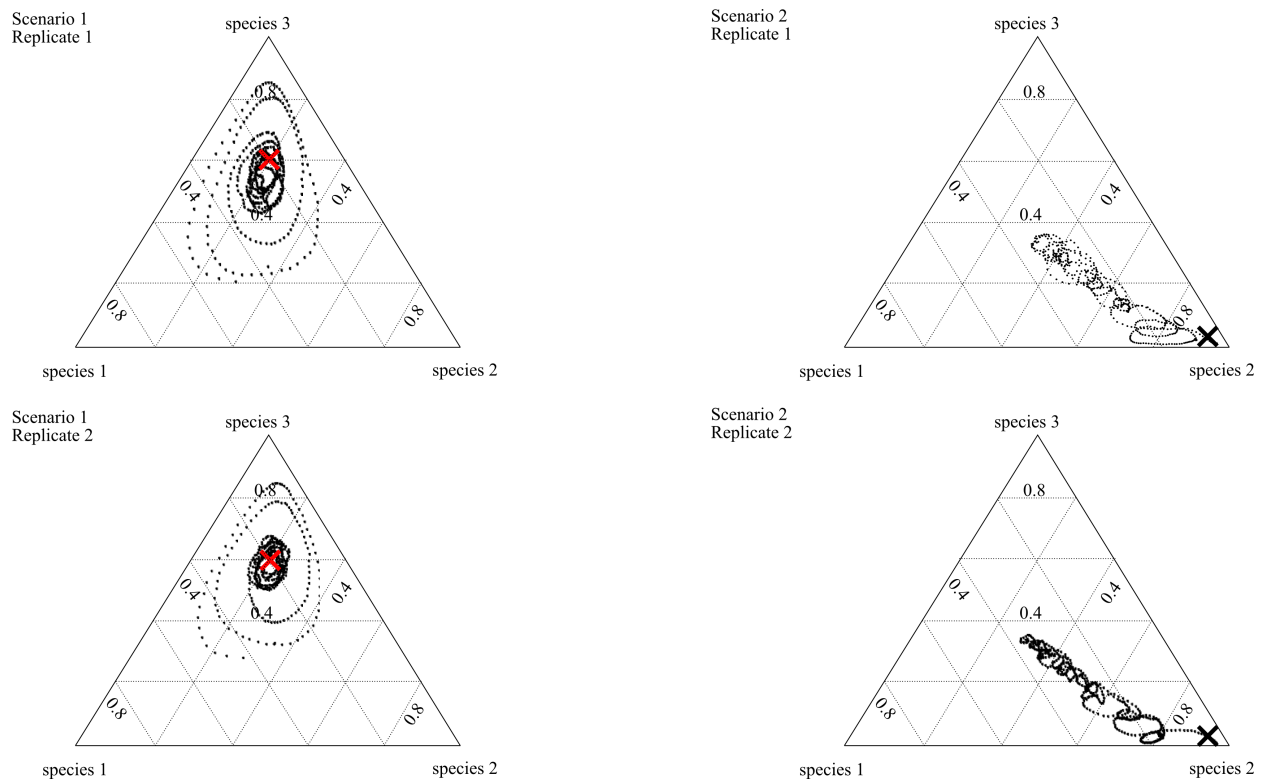


Figure 5.5: Ternary plots of the replicates of the two scenarios.

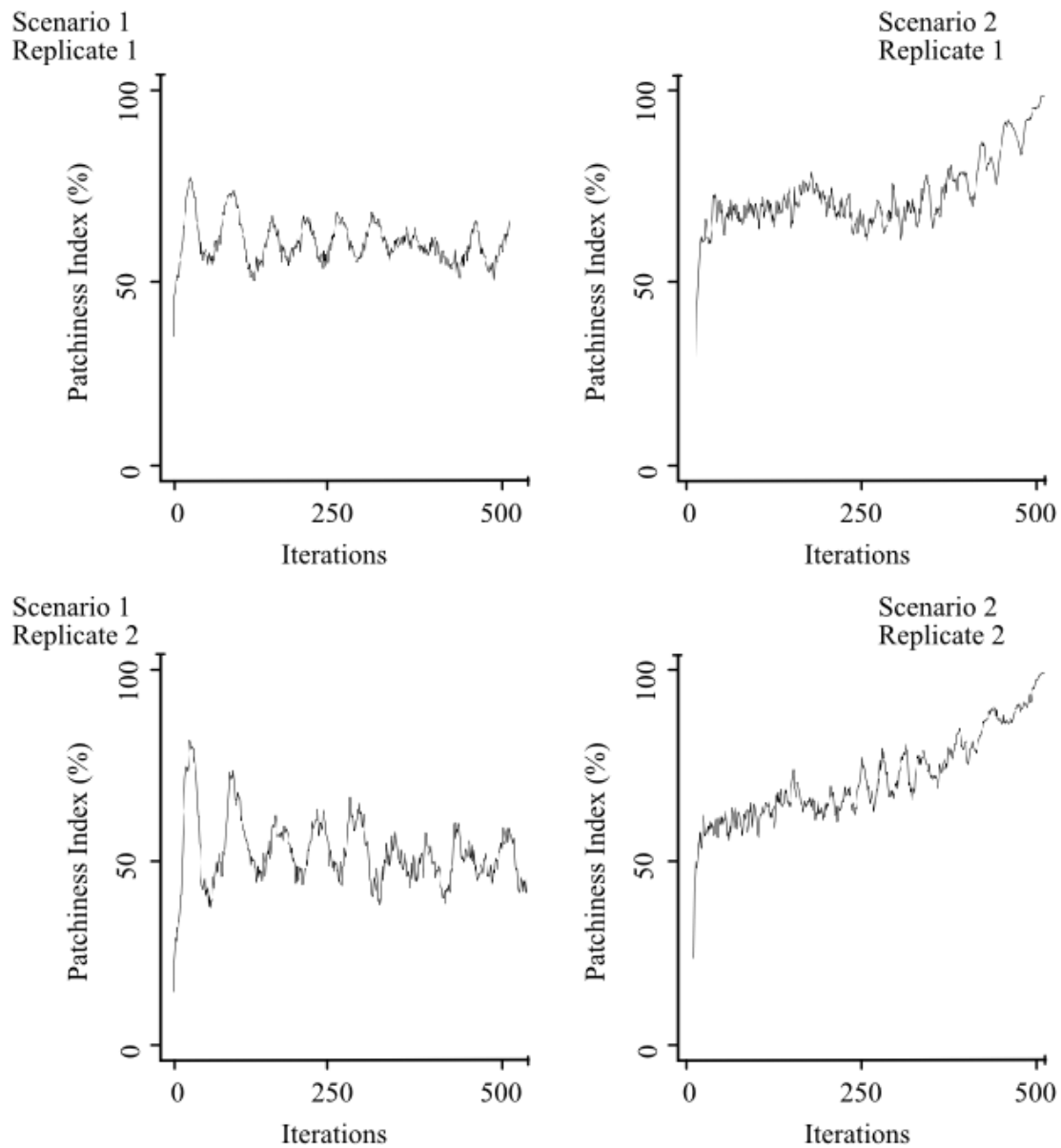


Figure 5.6: Patchiness indices of replicates of the two scenarios.

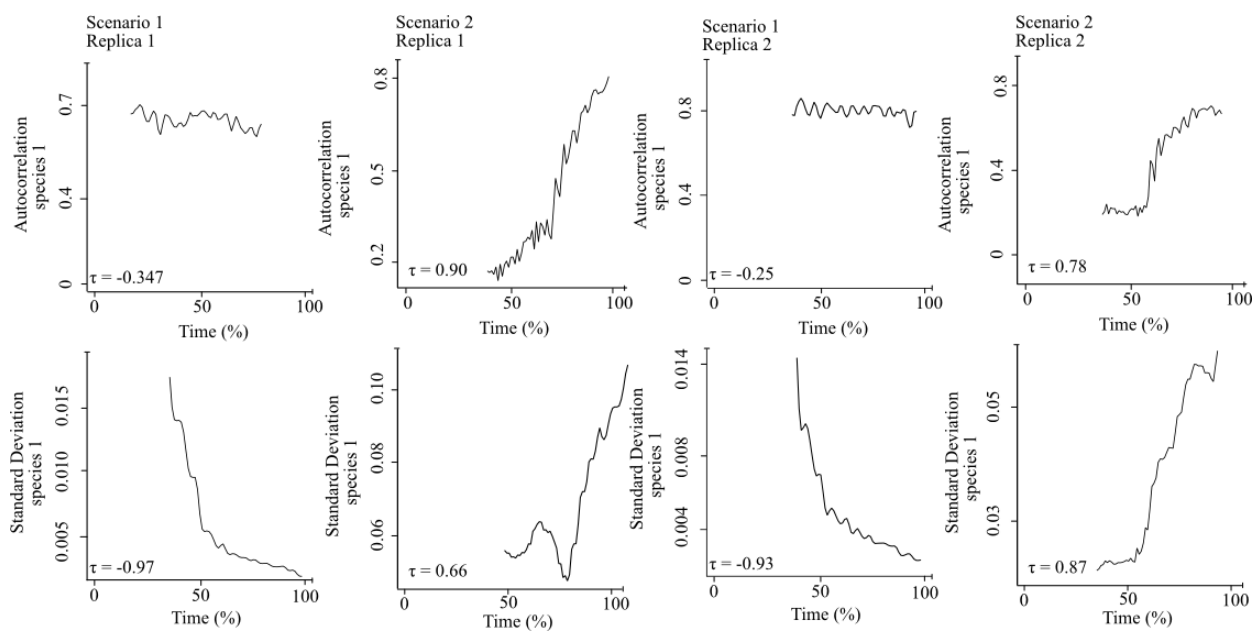


Figure 5.7: Early warning signals (autocorrelation and variance) calculated on the detrended relative log-abundance of species 1 with bandwidth 6,5% and window size 35% on the replicates of the two scenarios.

Chapter 6

Final considerations.

6.1 General perspective on resilience assessment

The conceptual underpinning of resilience in its various theoretical aspects is well established. Beyond resistance - the ability of a system to remain within an equilibrium zone in spite of external pressures - it characterizes capacity and response time for the system to return to its original equilibrium after a disturbance (Elliott *et al.*, 2007). Within a basin of attraction, the response is mostly linear, however shifts to alternate stable states may occur suddenly, and at times a point of no return, a threshold, is observed that is difficult to detect mathematically and modelling-wise, thus subject to a significant error (Andersen *et al.*, 2009; Elliott *et al.*, 2007; Hefley *et al.*, 2013). In a variety of cases signals might detect an upcoming critical transition (Boettiger *et al.*, 2013; van Nes *et al.*, 2007). Measuring resilience is challenging and recent research focusses on rendering the concept operational by using statistical indicators, early warning signals (EWS), with the capacity of indicating a system is at risk of being forced out of its basin of attraction (van Hes and Scheffer, 2007).

The first case study of this PhD thesis used recovery time as a resilience assessment tool (Andersen *et al.*, 2009). It aimed to measure the response time at population and community level of the macrobenthic community in a coastal lagoon after its artificial opening. Theoretically ecosystems with low resilience show a slower recovery rate compared to highly stable ecosystems (Scheffer *et al.*, 2009; Simon *et al.*, 2003). A key finding of the study was that despite the management intervention, most macrobenthic *taxa* did not show any sign of recovery. Moreover the poor diversity and dominance of opportunistic species demonstrated the lagoon underwent a regime shift prior to the current monitoring program. Overall the study reinforced three priorities and key hurdles related to effective resilience assessment. First the importance of identifying thresholds and anticipating regime shifts: forecasting critical transitions towards an alternate stable state is essential as once occurred reversal and re-establishment of good environmental status is challenging to re-establish (Newton and Mudge, 2005). In the case of the coastal lagoon an improvement in its state will require more intense management actions: deepening the inlet channel and a more frequent opening are suggested as possible actions to achieve a more effective flushing and improvement of the water quality (Elliott *et al.*, 2007). Secondly the recovery time of macrobenthos might be subject

to extended time-lags, especially if the ecosystem shifted to an alternate stable state: extending the period of the open connection to the sea as practiced elsewhere, might have allowed at least partial recovery of the species richness (Pereira Coutinho *et al.*, 2012). A final key finding was that the spatiotemporal resolution of the monitoring program defines the quality of the resilience assessment: previous sampling of the coastal lagoon dates back to the 70'ties and a more continuous monitoring might have enabled early detection and possible prevention of the regime shift.

The following studies of this thesis, focus on a different aspect of resilience assessment: statistical indicators as advanced warning of upcoming shifts. Here we apply two indicators frequently used as EWS to measure resilience: autocorrelation and variance (Dakos *et al.*, 2008; Scheffer *et al.*, 2009; van Nes and Scheffer 2007). Successful empirical examples of EWS in a variety of environments highlight their potential, and their effectiveness was tested in large scale field surveys, laboratory experiments and models (Carpenter *et al.*, 2011; Drake and Griffen 2010; Benincà *et al.*, 2009). Two aspects contribute to their attractiveness as resilience assessment tools: their applicability across disciplines and their relatively easiness of calculation, provided appropriate statistical requirements are met (Boettiger *et al.*, 2013; Dakos *et al.*, 2008). The EWS case studies of this thesis address two main challenges of the current state of the art of EWS research namely their applicability in marine and estuarine ecosystems and their behaviour involving regimes with cyclic dynamics. Furthermore the methodological constraints of EWS are tackled and possible pathways to overcome those bottlenecks are tested by innovative methods.

6.2 Limitating EWS challenges

A factor influencing EWS detection relates to the boundaries of the ecosystem. Arguably, more accurate results could be yielded in systems with identifiable limits such as lakes or intertidal pools on rocky shores in comparison to large-scale open systems like oceans or coral reefs (Dakos *et al.*, in press). Previously EWS detection in marine regime shifts occurred indeed in ecosystems constrained within physical boundaries, or focused on communities with reduced mobility like macrobenthos of the intertidal zone (Lindegren *et al.*, 2012; Hewitt and Thrush 2010). However case studies in open seas do exist, while being sparse e.g. EWS were detected in a series of collapses of Crustacean fisheries in the Gulf of Alaska and Bering Sea and in a marine regime shifts in

the North Atlantic (Beaugrand 2004; Litzow *et al.*, 2013; Litzow *et al.*, 2014). Our findings of increasing EWS trends in both a semi-open system like the Solent estuary and an open system like the central North Sea reinforces the successful application of EWS in the marine world. Moreover while both studies cover different types of dynamic ecosystem changes - irreversible decline of a population vs a regime shift - EWS showed increasing trends in both cases suggesting that the underlying mechanisms driving shifts might not be most essential for EWS detection.

Certain systems are characterized by intrinsic cycles e.g. species abundance in cyclic competitive hierarchies and predator prey interactions e.g. whelk-lobster in marine benthic communities (Barkai and Mcquaid 1998; Freat and Abraham 2001). Also the trends of abiotic drivers are commonly characterized by cyclicity for example climate phenomena such as the decadal North Atlantic Oscillation and glaciations with a periodicity of 100 Kyears (Petit *et al.*, 1999). While dynamic cyclicity is expected to exhibit increasing EWS trends when resilience is lost, not a lot of research investigates this issue (Scheffer *et al.*, 2009). Rare examples include: increasing EWS trends detected upcoming bifurcations in cyclic two-species predator-prey models and were also demonstrated in foodweb models of marine plankton characterized with cyclic dynamics (Benincà *et al.*, 2009; Chisolm and Filotas 2009). Two case studies of this thesis encourage building further experience with EWS as tools to assess loss of resilience of cyclostationary. In the trend of French oyster landings low frequency sine-waves were detected linked to NAO and while the time series was not sufficiently long to firmly assess its stability on long scale, EWS remained overall stable. In the study based on a modelling approach with three species interacting in a competitive loop according to 'rock-paper-scissors' (RPS) non-transitive hierarchies, EWS effectively reflected the contrasting stability of the two scenarios.

6.3 Key factors influencing EWS reading

The precision of EWS is influenced by time scale and the choice of the indicators (Boettiger *et al.*, 2013). Longer time series and frequent observations over time (e.g. yearly vs. monthly) provide more accurate EWS readings (Lenton *et al.*, 2012). In this perspective, the design of the monitoring program is crucial: long and high resolution sampling protocols add much value for resilience assessment (Dakos *et al.*, 2012). An encouraging pathway is to take advantage of lengthy time

series available in the framework of long time ongoing monitoring programmes like demonstrated by the case study of regime shift in the North Sea, based on monthly surveys initiated in 1957. It allowed in depth methodological testing assessing the robustness and significance of EWS and, 12 years prior to the regime shift, the loss of resilience was significantly detected. High frequency measurements have become more and more a routine in recent years, and thus offer novel opportunities for EWS testing especially for the marine environment (Blain *et al.*, 2004). In addition high resolution data tend to be more suitable for manipulation and experimentation, a crucial issue to generate accurate replicates (Dakos *et al.*, in press). Specifically for cyclic phenomena, time scale is a key factor as the number of residual harmonic fluctuations needs to be sufficient to generate an autocorrelation trend within the limits of the sliding window used for its calculation (Wouters *et al.*, 2013). The measure upon which EWS is applied is also essential: variables with fast turnover rate simply respond swifter to changes in external stressors; measures of primary production (e.g. phytoplankton) or nutrient recycling might be therefore more appropriate than the abundance of species with long life cycles (e.g. macrobenthos). While in the Solent case study macrobenthos did generate EWS in short time scale, using phytoplankton parameters as variables provided a more timely warning. Normalisation and standardisation of the raw data proved strategic as it allowed for a unitless comparison. The choice of the EWS indicators itself is essential: variance can be estimated more precisely as observations are more frequent, while autocorrelation is estimated more accurately as the time span increases (Brock and Carpenter 2012). However this can be partially overcome by calculating multiple EWS (Lindeberg *et al.*, 2012). Other operational hurdles of EWS application relate to statistical dependence and robustness (Boettiger *et al.*, 2013). The restricted degrees of freedom or unpredictable distribution (e.g. limited observations, patchiness) are statistically challenging. For example, in the case of sliding windows, sequential values of autocorrelation belong a priori to the same population, and are thus not statistically independent. Furthermore, in order to generate an appropriate estimate of the population, spatial sampling should be done with caution and take into account possible aggregations of units. Robustness is also particularly challenging, as slowing down is not assessed by absolute values but by trends that in some cases consist of differences in the order of magnitude or 0.2 (Lenton *et al.*, 2012). In case of autocorrelation, the indicator values vary between -1 and 1. Negative values are not unusual in

biology; in the case of a shift in dominance of phytoplankton species the values increased from -1 to 0 (Carpenter *et al.*, 2009). Methodologically, a negative sign of lag-1 autocorrelation reflects that consecutive residuals have opposite signs with positive residuals being almost always followed by negative residuals and vice versa. Despite at times being minor, advanced methodological testing in case of our study on the North Sea regime shift, proved that the trend change was robust. Small deviations in EWS should therefore not be omitted by managers. The use of a sliding window limits the robustness of EWS trends as there is a data overlap between the samples. This drawback can be overcome by dividing the time series into independent blocks, in case of lengthy time series as it was proven effective to the monthly averages in the North Sea phytoplankton analyses. Also the use of a window for EWS calculation implies that exceptional peaks in the data carry a significant weight on a succession of EWS values, which might create difficulties to interpret any sudden step changes. This effect is amplified with the use of larger window sizes and we therefore recommend sensitivity analyses to test for the variation of EWS trends under varying window sizes. It is therefore advised to smooth statistical outliers in the statistical distribution of the data prior to EWS assessment by applying an appropriate transformation, e.g. log or square root, to achieve trends less affected by the weight of singular points. To navigate away from unclear EWS signals and possible difficulties in interpreting trends, it is suggested to back-up EWS assessment with site-specific ecosystems models if available.

6.4 Insights on future resilience assessment in marine research

A prime recommendation is to invest considerably more research in aquatic EWS studies with special attention to the variety of marine or estuarine habitats such as coral reefs, intertidal flats, open ocean, coastal lagoons etc. Case studies covering the different habitats would allow to initiate a database of characteristic marine, coastal and estuarine EWS trends and establish baseline values eventually leading to EWS signatures. The database could ultimately allow a ranking of reefs, lakes or other ecosystems according to their resilience (Dakos *et al.*, 2015). A second recommendation put forward by this thesis is to extend the field of EWS application in cyclical conditions, which are pervasive in all ecosystems. Biological systems are often characterized by intrinsic cycles caused by the interaction between fast and slow variables and, while under such conditions no formal

bifurcation points are crossed, indicators of CSD may be observed as resilience is lost as shown in the case study on cyclic competitive hierarchies. A crucial step in enhancing the application of EWS in aquatic environments consists of familiarising ecosystem managers and stakeholders with EWS research and their potential in terms of detecting regime shift in advance while highlighting the relative easiness of their calculation. Finally EWS are particularly promising as resilience assessment tools in the light of novel monitoring techniques generating real time, high resolution data on large geographical scales such as aerial photographs or satellite images and monitoring buoys.

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Chapter 7

Supplementary material.

Automaton Software of Chapter 5

```
## Load .R version 3.2.7.
## Load Matrix, Matlab sub-packages
## Introduce parameters in Parameter input zone
## within the initialisation area (1):
## Example for Scenario 1 of paper:
## f12<-4/3
## f23<-0.5476
## r12<-0.7143
## r23<-0.78261
## r31<-0.6383
## The following \D" is a \damping" factor, equivalent to a \global response time"
## When set to 1, each and any point skips an iteration point, hence every thing stands still
## When D=0, action occurs at each point at every iteration. In between, iterations are missed out
## at randomly selected points, in the proportion of 1-D
## D<-0
## Gives the following matrix:
## 0 f1,2r1,2 f1,3r1,3
## f2,1r2,1 0 f2,3r2,3
## f3,1r3,1 f3,2r3,2 0
## gives approx. (rounding off.'s...):
## 0 0.95 0.40
## 0.38 0 0.43
## 0.71 0.12 0
## Run the initialisation of area (1) :Will produce a random input
## if skipping the sequence indicated "skip", otherwise
## will produce an exact checker board (what we did in all
## the illustrated cases)
## Conventions will become R=1 (Blue), C= 2 (Yellow), S= 3 (Brown)
## after the first complete run
## The sequences can be run (2) as many times as desired, manually if the command
## for (h in 1:500)
## is neutralised by a ##, and if the corresponding '}' at the end of the sequence to repeat
## is also neutralised
## Then, when manual iteration, the step-out control read outs
## can be watched every iteration, if desired
## (1) initialisation
## To clean the data base, before running a case, so that no left-over from any
## previous runs
ran<-matrix(0,729,27)
ran2<-matrix(0,729,27)
Count<-matrix(0,500,3)
colnames(Count)<-c("Species 1","Species 2","Species 3")
n<-c(1,1,1)
## Parameter input zone; in red: the parameter to change according to needs
##F12<-4/3
##f12<-4/3
##F23<-0.5476
##f23<-0.5476
F12<-1
f12<-1
```

```

F23<-1
f23<-1
r12<-1
r23<-1
r31<-1
D<-0
## Choose the correct output number for patches, here, example is Patch 2
## End of Parameter input zone
## Continued initialisation, using inputed parameters appropriately
f31<-(3-f12-f23)
f21<-f12
f32<-f23
f13<-f31
r13<-(1-r31)
r32<-(1-r23)
r21<-(1-r12)
f<-rbind(c(0,f12,f13),c(f21,0,f23),c(f31,f32,0))
r<-rbind(c(0,r12,r13),c(r21,0,r23),c(r31,r32,0))
q<-cbind(c(0,0,0),c(0,0,0),c(0,0,0))
km1seed<-cbind(c(1,2,3),c(2,3,1),c(3,1,2))
## checker board
km1<-repmat(km1seed,9)
kseed<-cbind(c(2,3,1),c(3,1,2),c(1,2,3))
k<-repmat(kseed,9)
kp1seed<-cbind(c(3,1,2),c(1,2,3),c(2,3,1))
kp1<-repmat(kp1seed,9)
G<-rbind(km1,k,kp1)
a<-rbind(G,G,G,G,G,G,G,G)
Core<-zeros(27,27)
time<-0
## Skip until \next" if a pure checker board is selected to start
## not random initialisation, otherwise random
####for (k in 1:27){for (j in 1:27){for (i in (27*k-26):(27*k)){runif
####(1)-> ran[i,j])}}
####ran<-ran*3
####for (k in 1:27){for (j in 1:27){for (i in (27*k-26):(27*k))
####(ifelse(ran[i,j]<1,1,ifelse(ran[i,j]>2,3,2)))->a[i,j])}}
## If not checkerboard, import values for start-up:
##as.matrix(read.table("... 4d/b0"))->a
## next
for (i in 1:27){for (j in 1:27){a[i+351,j]->Core[i,j]}}
imagesc(Core)
Patch<-matrix(NA,500,2)
colnames(Patch)<-c("Iter","pct patch")
## Initialisation done. Iterations to start here
## key for the number of iteration, adjust the underlined number,
## here 500: for (h in 1:500)
for (h in 1:500){
c1<-0
c2<-0
c3<-0
for (k in 1:27) {p1<-zeros(27*k,27)}
b<-p1
p2<-p1

```

```

t<-p2+1
## also good as t<-p2, but avoids a division by zero
## unchanged outcome, as it will still result in zero's
## in buffer border zones (see further comment)
## Core equations for probability at each point (long computing time)
## To save on computing time, equations assume that the last choice, after testing
## This is true anywhere away from the immediate border of the model
## where zero species are initialised. This computer time saving introduces
## a border asymmetry, sensitive only for highly undecisive encounters
## at or close to 50/50 chance of domination
for (k in 2:26){for (j in 2:26){for (i in (27*k-25):(27*k-1)){
  (ifelse(a[i,j]==1,
    (ifelse(a[i-1,j]==1,n[1],ifelse(a[i-1,j]==3,f[3,1]*r[1,3],f[1,2]*r[1,2]))
    +ifelse(a[i-27,j]==1,n[1],ifelse(a[i-27,j]==3,f[3,1]*r[1,3],f[1,2]*r[1,2]))
    +ifelse(a[i,j+1]==1,n[1],ifelse(a[i,j+1]==3,f[3,1]*r[1,3],f[1,2]*r[1,2]))
    +ifelse(a[i+1,j]==1,n[1],ifelse(a[i+1,j]==3,f[3,1]*r[1,3],f[1,2]*r[1,2]))
    +ifelse(a[i+27,j]==1,n[1],ifelse(a[i+27,j]==3,f[3,1]*r[1,3],f[1,2]*r[1,2]))
    +ifelse(a[i,j-1]==1,n[1],ifelse(a[i,j-1]==3,f[3,1]*r[1,3],f[1,2]*r[1,2]))
    ),0)
  +ifelse(a[i,j]==2,
    (ifelse(a[i-1,j]==1,f[1,2]*r[1,2],0)
    +ifelse(a[i,j+1]==1,f[1,2]*r[1,2],0)
    +ifelse(a[i-27,j]==1,f[1,2]*r[1,2],0)
    +ifelse(a[i+1,j]==1,f[1,2]*r[1,2],0)
    +ifelse(a[i,j-1]==1,f[1,2]*r[1,2],0)
    +ifelse(a[i+27,j]==1,f[1,2]*r[1,2],0)
    ),0)
  +ifelse(a[i,j]==3,
    (ifelse(a[i-1,j]==1,f[3,1]*r[1,3],0)
    +ifelse(a[i,j+1]==1,f[3,1]*r[1,3],0)
    +ifelse(a[i-27,j]==1,f[3,1]*r[1,3],0)
    +ifelse(a[i+1,j]==1,f[3,1]*r[1,3],0)
    +ifelse(a[i,j-1]==1,f[3,1]*r[1,3],0)
    +ifelse(a[i+27,j]==1,f[3,1]*r[1,3],0)
    ),0))>->p1[i,j]))
for (k in 2:26){for (j in 2:26){for (i in (27*k-25):(27*k-1)){
  (ifelse(a[i,j]==2,
    (ifelse(a[i-1,j]==2,n[2],ifelse(a[i-1,j]==1,f[1,2]*r[2,1],f[2,3]*r[2,3]))
    +ifelse(a[i-27,j]==2,n[2],ifelse(a[i-27,j]==1,f[1,2]*r[2,1],f[2,3]*r[2,3]))
    +ifelse(a[i,j+1]==2,n[2],ifelse(a[i,j+1]==1,f[1,2]*r[2,1],f[2,3]*r[2,3]))
    +ifelse(a[i+1,j]==2,n[2],ifelse(a[i+1,j]==1,f[1,2]*r[2,1],f[2,3]*r[2,3]))
    +ifelse(a[i+27,j]==2,n[2],ifelse(a[i+27,j]==1,f[1,2]*r[2,1],f[2,3]*r[2,3]))
    +ifelse(a[i,j-1]==2,n[2],ifelse(a[i,j-1]==1,f[1,2]*r[2,1],f[2,3]*r[2,3]))
    ),0)
  +ifelse(a[i,j]==3,
    (ifelse(a[i-1,j]==2,f[2,3]*r[2,3],0)
    +ifelse(a[i,j+1]==2,f[2,3]*r[2,3],0)
    +ifelse(a[i-27,j]==2,f[2,3]*r[2,3],0)
    +ifelse(a[i+1,j]==2,f[2,3]*r[2,3],0)
    +ifelse(a[i,j-1]==2,f[2,3]*r[2,3],0)
    +ifelse(a[i+27,j]==2,f[2,3]*r[2,3],0)
    ),0)
  +ifelse(a[i,j]==1,
    (ifelse(a[i-1,j]==2,f[1,2]*r[2,1],0)

```

```

+ifelse(a[i,j+1]==2,f[1,2]*r[2,1],0)
+ifelse(a[i-27,j]==2,f[1,2]*r[2,1],0)
+ifelse(a[i+1,j]==2,f[1,2]*r[2,1],0)
+ifelse(a[i,j-1]==2,f[1,2]*r[2,1],0)
+ifelse(a[i+27,j]==2,f[1,2]*r[2,1],0)
),0))>p2[i,j]))
## for fast computing, can avoid this step and use
## the complement to 1 of the third probability
## This short-cut a small asymmetry, arising from the border conditions
## for (k in 2:26)(for (j in 2:26)(for (i in (27*k-25):(27*k-1)) (
## (ifelse(a[i,j]==3,
## (ifelse(a[i-1,j]==3,n[2],ifelse(a[i-1,j]==1,f[1,3]*r[3,1],f[3,2]*r[3,2]))
## +ifelse(a[i-27,j]==3,n[2],ifelse(a[i-27,j]==1,f[1,3]*r[3,1],f[3,2]*r[3,2]))
## +ifelse(a[i,j+1]==3,n[2],ifelse(a[i,j+1]==1,f[1,3]*r[3,1],f[3,2]*r[3,2]))
## +ifelse(a[i+1,j]==3,n[2],ifelse(a[i+1,j]==1,f[1,3]*r[3,1],f[3,2]*r[3,2]))
## +ifelse(a[i+27,j]==3,n[2],ifelse(a[i+27,j]==1,f[1,3]*r[3,1],f[3,2]*r[3,2]))
## +ifelse(a[i,j-1]==3,n[2],ifelse(a[i,j-1]==1,f[1,3]*r[3,1],f[3,2]*r[3,2]))
## ),0)
## +ifelse(a[i,j]==1,
## (ifelse(a[i-1,j]==3,f[3,1]*r[3,1],0)
## +ifelse(a[i,j+1]==3,f[3,1]*r[3,1],0)
## +ifelse(a[i-27,j]==3,f[3,1]*r[3,1],0)
## +ifelse(a[i+1,j]==3,f[3,1]*r[3,1],0)
## +ifelse(a[i,j-1]==3,f[3,1]*r[3,1],0)
## +ifelse(a[i+27,j]==3,f[3,1]*r[3,1],0)
## ),0)
## +ifelse(a[i,j]==2,
## (ifelse(a[i-1,j]==3,f[3,2]*r[3,2],0)
## +ifelse(a[i,j+1]==3,f[3,2]*r[3,2],0)
## +ifelse(a[i-27,j]==3,f[3,2]*r[3,2],0)
## +ifelse(a[i+1,j]==3,f[3,2]*r[3,2],0)
## +ifelse(a[i,j-1]==3,f[3,2]*r[3,2],0)
## +ifelse(a[i+27,j]==3,f[3,2]*r[3,2],0)
## ),0))>p3[i,j]))
for (k in 2:26)(for (j in 2:26)(for (i in (27*k-25):(27*k-1)) (
(ifelse(a[i,j]==1,
(ifelse(a[i-1,j]==1,1,ifelse(a[i-1,j]==3,f[3,1],f[1,2]))
+ifelse(a[i-27,j]==1,1,ifelse(a[i-27,j]==3,f[3,1],f[1,2]))
+ifelse(a[i,j+1]==1,1,ifelse(a[i,j+1]==3,f[3,1],f[1,2]))
+ifelse(a[i+1,j]==1,1,ifelse(a[i+1,j]==3,f[3,1],f[1,2]))
+ifelse(a[i+27,j]==1,1,ifelse(a[i+27,j]==3,f[3,1],f[1,2]))
+ifelse(a[i,j-1]==1,1,ifelse(a[i,j-1]==3,f[3,1],f[1,2]))
),0)
+ifelse(a[i,j]==2,
(ifelse(a[i-1,j]==2,1,ifelse(a[i-1,j]==1,f[1,2],f[2,3]))
+ifelse(a[i-27,j]==2,1,ifelse(a[i-27,j]==1,f[1,2],f[2,3]))
+ifelse(a[i,j+1]==2,1,ifelse(a[i,j+1]==1,f[1,2],f[2,3]))
+ifelse(a[i+1,j]==2,1,ifelse(a[i+1,j]==1,f[1,2],f[2,3]))
+ifelse(a[i+27,j]==2,1,ifelse(a[i+27,j]==1,f[1,2],f[2,3]))
+ifelse(a[i,j-1]==2,1,ifelse(a[i,j-1]==1,f[1,2],f[2,3]))
),0)
+ifelse(a[i,j]==3,
(ifelse(a[i-1,j]==3,1,ifelse(a[i-1,j]==2,f[2,3],f[3,1]))
+ifelse(a[i-27,j]==3,1,ifelse(a[i-27,j]==2,f[2,3],f[3,1]))

```

```

+ifelse(a[i,j+1]==3,1,ifelse(a[i,j+1]==2,f[2,3],f[3,1]))
+ifelse(a[i+1,j]==3,1,ifelse(a[i+1,j]==2,f[2,3],f[3,1]))
+ifelse(a[i+27,j]==3,1,ifelse(a[i+27,j]==2,f[2,3],f[3,1]))
),0))>->t[i,j]))
P1<-p1/t
P2<-p2/t
P3<-(1-(P1+P2))
## PP3<-p3/t
## this leaves zero probability on the edges, de-facto reducing the cube
## by one rank all around, but allowing the equations to function
##sets of random numbers
for (k in 1:27){for (j in 1:27){for (i in (27*k-26):(27*k))(runif(1)->ran[i,j]))}
for (k in 1:27){for (j in 1:27){for (i in (27*k-26):(27*k))(runif(1)->ran2[i,j]))}
## new sets of values (b) in cells, taking probabilities, and using generated random numbers
for (k in 2:26){for (j in 2:26){for (i in (27*k-25):(27*k-1))
+(ifelse(ran[i,j]<D,a[i,j],ifelse(ran2[i,j]<P1[i,j],1,ifelse(ran2[i,j]>(P1[i,j]+P3[i,j]),2,3)))->b[i,j]))}
## thus zero's on the borders, ie none of the species which can generate a probability.
## De facto, on the edges, half inner dpace is used
## instead of an ordered resetting of values, one can use a random approach, as
## in the following
## for (k in 2:26){for(j in sample(2:26)){for (i in sample((27*k-25):(27*k-1)))
+(ifelse(ran[i,j]<D,a[i,j],ifelse(ran2[i,j]<P1[i,j],1,ifelse(ran2[i,j]>(P1[i,j]+P3[i,j]),2,3)))->b[i,j]))}
## Outcome not much affected
for (j in 2:26){for (i in 2:728){ifelse(b[i,j]==1,(1+c1),c1)->c1))
for (j in 2:26){for (i in 2:728){ifelse(b[i,j]==2,1+c2,c2)->c2))
for (j in 2:26){for (i in 2:728){ifelse(b[i,j]==3,1+c3,c3)->c3))
## c1+c2+c3 totals up to 15625 ie the inside 25X25X25 matrix
time<-time+1
Count[time,<-c(c1,c2,c3)
a<-b
l<-0
for (i in 1:27){for (j in 1:27){a[i+351,j]->Core[i,j])}
## Image of the middle cross-section, easy to check out
## Not compulsory
imagesc(Core)
## Local dampening of frequency
##f12<-F12*(1-h/500)
##
f31<-(3-f12-f23)
f21<-f12
f32<-f23
f13<-f31
f<-rbind(c(0,f12,f13),c(f21,0,f23),c(f31,f32,0))
p<-0
for(i in 3:26){for(j in 2:26){ifelse(Core[(i-1),j]==Core[i,j],1+p,0+p)->p))
Patch[h,2]<-p/600*100
Patch[h,1]<-h
print(Count[time,])
print(h)
}
time
Count[time,]
## the following core equations, used instead of those in the above
## programme will avoid border asymmetries, but will need longer

```

```

## computing time
## Core equations for probability at each point (long computing time)
for (k in (2:26))(for (j in (2:26))(for (i in ((27*k-25):(27*k-1)))(
  (ifelse(a[i,j]==1,
    (ifelse(a[i-1,j]==1,n[1],ifelse(a[i-1,j]==3,f[3,1]*r[1,3],ifelse(a[i-1,j]==2,f[1,2]*r[1,2],0)))
    +ifelse(a[i-27,j]==1,n[1],ifelse(a[i-27,j]==3,f[3,1]*r[1,3],ifelse(a[i-27,j]==2,f[1,2]*r[1,2],0)))
    +ifelse(a[i,j+1]==1,n[1],ifelse(a[i,j+1]==3,f[3,1]*r[1,3],ifelse(a[i,j+1]==2,f[1,2]*r[1,2],0)))
    +ifelse(a[i+1,j]==1,n[1],ifelse(a[i+1,j]==3,f[3,1]*r[1,3],ifelse(a[i+1,j]==2,f[1,2]*r[1,2],0)))
    +ifelse(a[i+27,j]==1,n[1],ifelse(a[i+27,j]==3,f[3,1]*r[1,3],ifelse(a[i+27,j]==2,f[1,2]*r[1,2],0)))
    +ifelse(a[i,j-1]==1,n[1],ifelse(a[i,j-1]==3,f[3,1]*r[1,3],ifelse(a[i,j-1]==2,f[1,2]*r[1,2],0)))
  ),0)
  +ifelse(a[i,j]==2,
    (ifelse(a[i-1,j]==1,f[1,2]*r[1,2],0)
    +ifelse(a[i,j+1]==1,f[1,2]*r[1,2],0)
    +ifelse(a[i-27,j]==1,f[1,2]*r[1,2],0)
    +ifelse(a[i+1,j]==1,f[1,2]*r[1,2],0)
    +ifelse(a[i,j-1]==1,f[1,2]*r[1,2],0)
    +ifelse(a[i+27,j]==1,f[1,2]*r[1,2],0)
  ),0)
  +ifelse(a[i,j]==3,
    (ifelse(a[i-1,j]==1,f[3,1]*r[1,3],0)
    +ifelse(a[i,j+1]==1,f[3,1]*r[1,3],0)
    +ifelse(a[i-27,j]==1,f[3,1]*r[1,3],0)
    +ifelse(a[i+1,j]==1,f[3,1]*r[1,3],0)
    +ifelse(a[i,j-1]==1,f[3,1]*r[1,3],0)
    +ifelse(a[i+27,j]==1,f[3,1]*r[1,3],0)
  ),0))>p1[i,j]))
for (k in (2:26))(for (j in (2:26))(for (i in ((27*k-25):(27*k-1)))(
  (ifelse(a[i,j]==2,
    (ifelse(a[i-1,j]==2,n[2],ifelse(a[i-1,j]==1,f[1,2]*r[2,1],ifelse(a[i-1,j]==3,f[2,3]*r[2,3],0)))
    +ifelse(a[i-27,j]==2,n[2],ifelse(a[i-27,j]==1,f[1,2]*r[2,1],ifelse(a[i-27,j]==3,f[2,3]*r[2,3],0)))
    +ifelse(a[i,j+1]==2,n[2],ifelse(a[i,j+1]==1,f[1,2]*r[2,1],ifelse(a[i,j+1]==3,f[2,3]*r[2,3],0)))
    +ifelse(a[i+1,j]==2,n[2],ifelse(a[i+1,j]==1,f[1,2]*r[2,1],ifelse(a[i+1,j]==3,f[2,3]*r[2,3],0)))
    +ifelse(a[i+27,j]==2,n[2],ifelse(a[i+27,j]==1,f[1,2]*r[2,1],ifelse(a[i+27,j]==3,f[2,3]*r[2,3],0)))
    +ifelse(a[i,j-1]==2,n[2],ifelse(a[i,j-1]==1,f[1,2]*r[2,1],ifelse(a[i,j-1]==3,f[2,3]*r[2,3],0)))
  ),0)
  +ifelse(a[i,j]==3,
    (ifelse(a[i-1,j]==2,f[2,3]*r[2,3],0)
    +ifelse(a[i,j+1]==2,f[2,3]*r[2,3],0)
    +ifelse(a[i-27,j]==2,f[2,3]*r[2,3],0)
    +ifelse(a[i+1,j]==2,f[2,3]*r[2,3],0)
    +ifelse(a[i,j-1]==2,f[2,3]*r[2,3],0)
    +ifelse(a[i+27,j]==2,f[2,3]*r[2,3],0)
  ),0)
  +ifelse(a[i,j]==1,
    (ifelse(a[i-1,j]==2,f[1,2]*r[2,1],0)
    +ifelse(a[i,j+1]==2,f[1,2]*r[2,1],0)
    +ifelse(a[i-27,j]==2,f[1,2]*r[2,1],0)
    +ifelse(a[i+1,j]==2,f[1,2]*r[2,1],0)
    +ifelse(a[i,j-1]==2,f[1,2]*r[2,1],0)
    +ifelse(a[i+27,j]==2,f[1,2]*r[2,1],0)
  ),0))>p2[i,j]))
for (k in (2:26))(for (j in (2:26))(for (i in ((27*k-25):(27*k-1)))(
  (ifelse(a[i,j]==3,

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(iffelse(a[i-1,j]==3,n[2],iffelse(a[i-1,j]==1,f[1,3]*r[3,1],iffelse(a[i-1,j]==2,f[3,2]*r[3,2],0)))
+iffelse(a[i-27,j]==3,n[2],iffelse(a[i-27,j]==1,f[1,3]*r[3,1],iffelse(a[i-27,j]==2,f[3,2]*r[3,2],0)))
+iffelse(a[i,j+1]==3,n[2],iffelse(a[i,j+1]==1,f[1,3]*r[3,1],iffelse(a[i,j+1]==2,f[3,2]*r[3,2],0)))
+iffelse(a[i+1,j]==3,n[2],iffelse(a[i+1,j]==1,f[1,3]*r[3,1],iffelse(a[i+1,j]==2,f[3,2]*r[3,2],0)))
+iffelse(a[i+27,j]==3,n[2],iffelse(a[i+27,j]==1,f[1,3]*r[3,1],iffelse(a[i+27,j]==2,f[3,2]*r[3,2],0)))
+iffelse(a[i,j-1]==3,n[2],iffelse(a[i,j-1]==1,f[1,3]*r[3,1],iffelse(a[i,j-1]==2,f[3,2]*r[3,2],0)))
),0)
+iffelse(a[i,j]==1,
(iffelse(a[i-1,j]==3,f[3,1]*r[3,1],0)
+iffelse(a[i,j+1]==3,f[3,1]*r[3,1],0)
+iffelse(a[i-27,j]==3,f[3,1]*r[3,1],0)
+iffelse(a[i+1,j]==3,f[3,1]*r[3,1],0)
+iffelse(a[i,j-1]==3,f[3,1]*r[3,1],0)
+iffelse(a[i+27,j]==3,f[3,1]*r[3,1],0)
),0)
+iffelse(a[i,j]==2,
(iffelse(a[i-1,j]==3,f[3,2]*r[3,2],0)
+iffelse(a[i,j+1]==3,f[3,2]*r[3,2],0)
+iffelse(a[i-27,j]==3,f[3,2]*r[3,2],0)
+iffelse(a[i+1,j]==3,f[3,2]*r[3,2],0)
+iffelse(a[i,j-1]==3,f[3,2]*r[3,2],0)
+iffelse(a[i+27,j]==3,f[3,2]*r[3,2],0)
),0))>->p3[i,j]))
for (k in (2:26))(for (j in (2:26))(for (i in ((27*k-25):(27*k-1)))(
(iffelse(a[i,j]==1,
(iffelse(a[i-1,j]==1,iffelse(a[i-1,j]==3,f[3,1],iffelse(a[i-1,j]==2,f[1,2],0)))
+iffelse(a[i-27,j]==1,iffelse(a[i-27,j]==3,f[3,1],iffelse(a[i-27,j]==2,f[1,2],0)))
+iffelse(a[i,j+1]==1,iffelse(a[i,j+1]==3,f[3,1],iffelse(a[i,j+1]==2,f[1,2],0)))
+iffelse(a[i+1,j]==1,iffelse(a[i+1,j]==3,f[3,1],iffelse(a[i+1,j]==2,f[1,2],0)))
+iffelse(a[i+27,j]==1,iffelse(a[i+27,j]==3,f[3,1],iffelse(a[i+27,j]==2,f[1,2],0)))
+iffelse(a[i,j-1]==1,iffelse(a[i,j-1]==3,f[3,1],iffelse(a[i,j-1]==2,f[1,2],0)))
),0)
+iffelse(a[i,j]==2,
(iffelse(a[i-1,j]==2,1,iffelse(a[i-1,j]==1,f[1,2],iffelse(a[i-1,j]==3,f[2,3],0)))
+iffelse(a[i-27,j]==2,1,iffelse(a[i-27,j]==1,f[1,2],iffelse(a[i-27,j]==3,f[2,3],0)))
+iffelse(a[i,j+1]==2,1,iffelse(a[i,j+1]==1,f[1,2],iffelse(a[i,j+1]==3,f[2,3],0)))
+iffelse(a[i+1,j]==2,1,iffelse(a[i+1,j]==1,f[1,2],iffelse(a[i+1,j]==3,f[2,3],0)))
+iffelse(a[i+27,j]==2,1,iffelse(a[i+27,j]==1,f[1,2],iffelse(a[i+27,j]==3,f[2,3],0)))
+iffelse(a[i,j-1]==2,1,iffelse(a[i,j-1]==1,f[1,2],iffelse(a[i,j-1]==3,f[2,3],0)))
),0)
+iffelse(a[i,j]==3,
(iffelse(a[i-1,j]==3,1,iffelse(a[i-1,j]==2,f[2,3],iffelse(a[i-1,j]==1,f[3,1],0)))
+iffelse(a[i-27,j]==3,1,iffelse(a[i-27,j]==2,f[2,3],iffelse(a[i-27,j]==1,f[3,1],0)))
+iffelse(a[i,j+1]==3,1,iffelse(a[i,j+1]==2,f[2,3],iffelse(a[i,j+1]==1,f[3,1],0)))
+iffelse(a[i+1,j]==3,1,iffelse(a[i+1,j]==2,f[2,3],iffelse(a[i+1,j]==1,f[3,1],0)))
+iffelse(a[i+27,j]==3,1,iffelse(a[i+27,j]==2,f[2,3],iffelse(a[i+27,j]==1,f[3,1],0)))
+iffelse(a[i,j-1]==3,1,iffelse(a[i,j-1]==2,f[2,3],iffelse(a[i,j-1]==1,f[3,1],0)))
),0))>->t[i,j]))
P1<-p1/t
P2<-p2/t
P3<-p3/t

```